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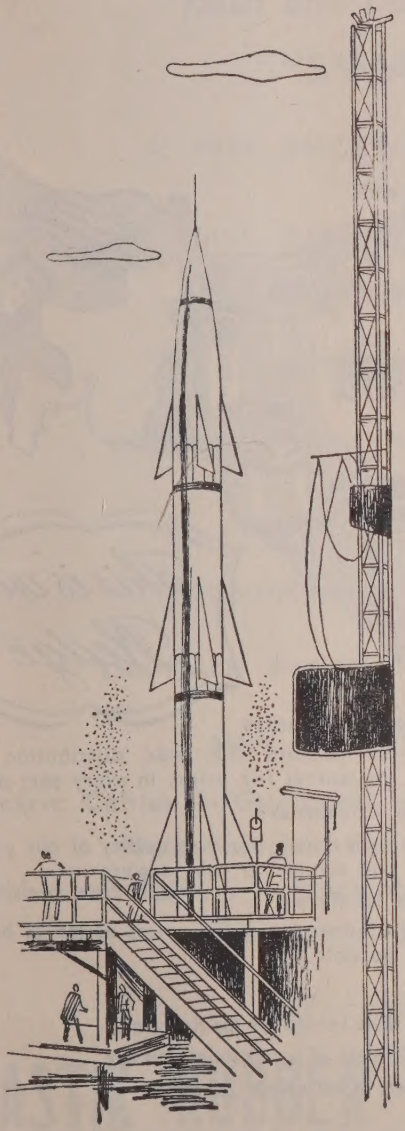

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K. 47 (e)

Antibiotics in the Control of Diseases Incited by Protozoa

AMONG the numerous diseases incited by pathogenic organisms in man, the protozoans occupy an important place. The protozoans in the blood and intestines form a characteristic group of which the *Plasmodium* species causing malaria and the spirochaetes responsible for syphilis have played an important part in the development of chemotherapy. Among the blood protozoans such forms as *Trypanosoma gambiense*, *T. equiperdum*, *T. cruzi* and also to some extent *Leishmania donovani* and *L. tropica*, have endemic distribution. On the other hand, amoebiasis incited by *Entamoeba histolytica*, and others like *Girardia intestinalis*, *Trichomonas vaginalis*, *T. hominis* are ubiquitous and are not known in some places due to lack of facilities for diagnosis. Amoebiasis and leishmaniasis are degenerative types of diseases often causing death of the host.

Until recently these protozoan diseases were treated by antiprotozoal chemicals such as quinine, atebrin, plasmoquine stibnal, Bayer 205, yatren, salvarsan and several arsenicals. These chemicals are highly toxic to human beings, so that the margin of safety is very narrow. For the first time, penicillin changed our concept of curing diseases incited by spirochaetes. As regards the other protozoan diseases, at first there was no systematic screening of the antibiotic producing organisms with antiprotozoal properties. The antibiotics developed for controlling bacterial and fungal diseases were tested against protozoan diseases particularly amoebiasis. Erythromycin and the tetracyclines proved quite effective in a number of instances. Ruibola *et al* used combinations or tetracycline and nystatin with good results for controlling *Entamoeba histolytica*. Seneca found the same effect, and also included novobiocin in the list. Fumagillin or fugillin, an antibiotic produced by *Aspergillus fumigatus* was shown by Macquiddy to be very effective in the treatment of amoebiasis. The work on finding an antibiotic with good amoebicidal effect and low toxicity is underway in many laboratories. Several new techniques for screening antibiotics *in vitro* by growing *E. histolytica* in artificial culture have been developed. *In vivo* studies often do not correlate with the results obtained *in vitro*. Even evaluation of the antiamoebic activity using a single laboratory animal like mouse or guinea pig is shown to be inadequate. Lynch *et al* pointed out that there should be at least two different types of laboratory animals for testing amoebicidal agents.

Among the new antibiotics recently reported as being effective against amoebiasis mention may be made of paramomycin or humatin described by Coffey *et al* and streptimidone by Kohberger *et al.*

The trypanosomes causing diseases such as sleeping sickness in tropical Africa have terrorized the people in the area. Puromycin (first designated as achromycin) reported by Porter *et al* was shown to have trypanocidal effect. Using *T. equiperdum* in mice, large number of antibiotics have been studied. Tetracycline and antimony complexes were shown to be effective by Lynch *et al.* Seneca and Ides showed that magnamycin inhibited *T. cruzi* in addition to *Leishmania donovani* at concentrations of 30 to 60 μg/ml. Nucleocidin produced by *Streptomyces calvus* was reported to be effective against *T. equiperdum.*

Trichomonas vaginalis is one of the organisms studied with a view to find an effective control measure. Trichomycin produced by *S. hachijoensis* was first shown to be very effective. As the disease is complicated by the association of *Candida albicans*, effective treatment is made more difficult. Nystatin combinations have been used for controlling moniliasis. Hori indicated that in addition to trichomycin, eurocidin, azomycin and aureothricin were also effective. Anisomycin reported by Sobin *et al* is stated to be effective against *Trichomonas*. Similarly favacid is reported to inhibit trichomonads. The antibiotic Hamycin recently reported from Hindustan Antibiotics is active against moniliasis and trichomonads in low concentrations. With the discovery of these new antiprotozoal antibiotics the prospects of combating amoebiasis, trypanosomiasis, leishmaniasis, etc., has become brighter.

An Antibiotic Related to Aburamycin

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AN antibiotic isolated from the broth of submerged cultures of a *Streptomyces* species isolated from soil samples collected near Pimpri, Poona, appeared to resemble M5-18903[1], an optical antipode of aburamycin[2]. Mycological studies on the organism producing the antibiotic and chemical studies of the antibiotic itself have revealed several interesting features, a brief account of which is presented here.

Aburamycin was isolated by Nishimura *et al*,[2] from a *Streptomyces* species closely related to, but distinct from *S. abikoensis* Okami. They described it as a new species, *S. aburaviensis*, its chief morphological character being the occurrence of grayish-white aerial mycelium and spores not being formed in spirals. A comparative account of the morphological and biochemical characters of *S. aburaviensis* and *S. abikoensis* is also given in their paper. Isolation of an optical antipode of aburamycin from a *Streptomyces* species isolated by Higgins from soil samples collected in Marshall islands, was reported by Gale *et al.*[1] A brief statement is made in their paper that the *Streptomyces* species is different from *S. aburaviensis* and reference is given to an unpublished paper by Higgins.

The *Streptomyces* species studied by us is distinct from *S. aburaviensis* in having a branched aerial mycelium bearing numerous closed spirals. The spores are ovate to ellipsoid. When first isolated, the submerged mycelium was yellowish-brown, and developed grayish aerial mycelium bearing the sporing structures in the form of closed spirals. After 4 or 5 subcultures, the sporing capacity was completely lost, and only the submerged vegetative mycelium remained, similar to the imperfect types known among *Streptomyces* species under prolonged artificial cultural conditions.

Production of the antibiotic was not affected by loss in sporing characters. Apart from the spiral nature of the spore bearing hyphae, the species under report (designated as culture 24-10) differed from *S. aburaviensis* as indicated in Table I.

The species is not described as a new species of *Streptomyces* as the type culture has lost the sporing characters. The organism was grown in submerged cultures on rotary shaker, incubated at 28°. Using a culture medium composed of cornsteep liquor 1 per cent, starch 2 per cent, ammonium sulphate 0.1 per cent and calcium carbonate 0.5 per cent, good yields of the antibiotic were obtained after 96 to 120 hr. fermentation.

The antibiotic was active against gram positive bacteria and *Mycobacterium phlei*. The range of activity when tested by serial dilution, was as follows (in μg/ml.) : *B. subtilis* 0.01, *Micrococcus pyogenes* var *aureus* 0.005 to 0.01., *Sarcina lutea* 0.01. *Mycobacterium phlei* 1.0. *Candida albicans Escherichia coli, Salmonella paratyphi* were not inhibited. The antibiotic was quite toxic to mice.

The antibiotic was isolated from the broth and in general characters appears to be the same as M5-18903 isolated by Gale *et al.*[1]

TABLE I. COMPARISON OF *S. aburaviensis* WITH CULTURE 24-10.

Media	*S. aburaviensis*	Culture 24-10
Czapeck's agar ..	Growth reverse, yellowish-brown. Soluble pigment dark yellowish-brown and aerial mycelium, well developed, velvetty, thick and white.	Growth poor, transparent submerged mycelium. Growth spreading type. Soluble pigment absent and colony reverse colourless.
Glucose asparagine agar	Growth grayish olive, thin, flat, reverse pale yellow. Soluble pigment at first, dull yellow, later becoming yellowish-brown. Aerial mycelium velvetty, almost white, slightly grayish.	Growth very poor, mycelium submerged and transparent. No soluble pigment.
Starch agar	Growth grayish, reverse yellow brown. Soluble pigment pale yellow brown. Aerial mycelium, grayish white.	Only submerged mycelium produced and colony reverse pale yellow green.
Starch hydrolysis ..	Weak	Moderate
Calcium malate agar ..	Growth pale yellow brown. Soluble pigment grayish yellow-brown. Aerial mycelium thin, white to grayish white.	Transparent submerged mycelium and growth spreading type. No soluble pigment and colony reverse white to pale yellow-green.
Gelatin liquifaction ..	Liquified and no soluble pigment.	Not liquified and no soluble pigment.
Nitrate reduction ..	Reduced.	Not reduced
Litmus milk	Coagulated and peptonized.	No coagulation or peptonization. Growth very poor.
Potato plug	Growth dull yellow to pale olive. No soluble pigment. Aerial mycelium white to light grey.	Growth good, with no aerial mycelium. Submerged greenish yellow. Dark-brown to black soluble pigment present.
Utilization of Carbohydrates.		
Glycerine	Good growth	Good growth
Starch	,,	,,
Glucose	,,	,,
Maltose	Fair growth	,,
Galactose	,,	Poor growth
Mannitol	No growth	,,
Arabinose	,,	Good growth
Raffinose	,,	,,
Lactose	,,	Poor growth
Saccharose	,,	,,

Purification

Even though M5-18903 and its optical antipode aburamycin were obtained in a crystalline form, we have not been able to obtain the antibiotic from culture 24-10 in a crystalline form. The filtered broth (4 l.) was adjusted to pH 6 and extracted with *n*-butanol. Evaporation of solvent *in vacuo*, and dilution with ether precipitated the antibiotic as a greenish yellow amorphous powder (2 g.). This was dissolved in ethyl acetate and washed thrice with 0.5 per cent sodium carbonate. The ethyl acetate layer was washed to neutral pH and evaporated *in vacuo*. Hexane trituration of the yellow residue gave a pale yellow greenish powder (1 g.), m. p. 212° (dec.)

The antibiotic was weakly acidic and could be extracted with 0.1*N* NaOH to give a pale, lemon yellow fluorescent solution, re-extractable on acidification with either chloroform or ethyl acetate. The compound gave a brownish green colour with ferric chloride. Both the procedures described by Gale *et al.*, and Nishimura *et al.*, did not yield a crystalline product. Nishimura's method, however, gave an amorphous substance with an indefinite m.p. 170-85.°

Characterization

The antibiotic (50 mg.) was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) at room temperature overnight. The pale yellow substance (44 mg.) obtained on pouring into crushed ice had m.p. 170-75.° The compound set to a mass of crystals in contact with methanol. Twice recrystallization from methanol gave pale yellow feathery needles, m.p. 206-07.° Analysis : C, 57.55 ; H, 6.3 per cent. The compound did not depress the m.p. of the acetate from M5-18903.

Tosylation with pyridine and *p*-toluene sulfonyl chloride at room temperature, or methylation with excess diazomethane failed to give any crystalline products.

The absorption spectrum of the antibiotic in 95 per cent ethanol was characterised by maxima at 229, 283, 316 and 420 mμ with $E^{1\%}_{1cm}$ 153, 520, 70 and 110 respectively.

Comparison of characteristics of the two acetates (Table II) leaves no doubt about their identity and it is not unreasonable to assume that the antibiotic from 24-10 is identical with M5-18903. Comparison of the parent antibiotics could not be made as the homogeniety of the antibiotic under study has not been rigorously established.

TABLE II. COMPARISON OF M5-18903 ACETATE WITH 24-10 ACETATE

	M5-18903 acetate	24-10 acetate
Melting point	206-07°	206-07°
Rotation in chloroform ..	$(\alpha)_D^{25}$ −24.13* (c=0.0063)	$(\alpha)_D^{25}$ −29.3 (c=0.0047)
Acetyl (%)	22.94	26.3
Absorption spectrum*:		
λmax. in mμ.	225, 267, 327, 365-70.	225, 267, 327, 365-70
$E^{1\%}_{1cm}$.	167, 376, 69, 27	183, 392, 70, 27.
Infra red spectra of the acetates of both compounds identical		

* Observed in this laboratory.

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We are indebted to Dr. R. M. Gale of Eli Lilly and Co. Indianapolis, Ind., U.S.A., for a sample of M5-18903.

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Occurrence of o-Hydroxyphenylacetic Acid in Abnormal Penicillin G Fermentations

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PHENYLACETIC acid, and phenylacetamide are the most commonly used precursors in penicillin fermentation for the production of benzylpenicillin. The concentrations, the rate of feeding, and the form in which the precursor is added depend on the strain, the fermentation medium, etc. Single addition of phenylacetamide (0.15 per cent), periodic addition of a solution of sodium or potassium phenylacetate, or continuous feeding of sucrose-sodium or potassium phenylacetate are some of the methods in commercial practice.

The precursor metabolism of the mould *Penicillium chrysogenum* is not clearly understood. The efficiency of utilization of the precursor in incorporating the benzyl moiety in penicillin molecule, varies very widely with strains and also depends on the fermentation conditions. Kleiner and Nagle[1] claimed that with the strain "New hybrid", 70 per cent of precursor was converted to penicillin, the efficiency of the precursor utilization being almost 100 per cent. In comparative fermentations, Wis. 51-20 converted at best only 50 per cent of the precursor into penicillin while the rest was probably oxidised. If phenylacetamide is used, the first stage is deamination to phenylacetic acid, which is rapid during the first 36-40 hrs. of the fermentation. It has also been suggested that the precursor may be oxidatively metabolised to the ultimate stage of carbon dioxide.[1,2] We have also found in the analyses of the precursor levels fed during the fermentation and the estimation of residual precursor at the end of fermentation, that a large part of it metabolised differently without being incorporated into the penicillin molecule. This utilization of the precursor by the mould seems to involve oxidative metabolism.

King and Hambly,[3] and Nishida[4] reported the isolation of *o*-hydroxyphenylacetic acid from the mother liquor after the isolation of first crystals of sodium or potassium penicillin G. Pan[5] identified it in crude preparations of penicillin and the mother liquors by colorimetric methods. However, the quantities isolated by these authors were very small, about 0.0008 per cent.

In the course of our studies on precursor utilization in industrial penicillin fermentations and the nature of impurities associated with first crystals of potassium penicillin we have isolated large quantities of *o*-hydroxyphenylacetic acid from some of the production batches. The acid was extracted along with penicillin from solvent extracts (butyl or amyl acetate) by aqueous buffers, or alkalies. The salts of *o*-hydroxyphenylacetic acid crystallized along with the penicillin salts in azeotropic distillation of the aqueous extracts and also in the precipitation of potassium penicillin by saturated potassium acetate. Potassium penicillin and *o*-hydroxyphenylacetic acid were separated from the resulting mixture by one of the following methods: (a) the mixture was washed with small quanti-

ties of ice-cold water, in which potassium *o*-hydroxyphenylacetate was less soluble so that potassium penicillin G could be separated ; (b) the penicillin in mixture was precipitated as procaine penicillin and the filtrate extracted with ether after acidification.

The potencies of potassium penicillin crystals from different batches under report are given in Table I. *o*-Hydroxyphenylacetic acid was completely extracted by potassium acetate and the potassium salt precipitated by saturated solution of potassium acetate. In a few batches the product crystallized from the potassium acetate extract itself. The potassium salt crystallized in colourless flakes (m.p. 245-50°) from hot water. The aqueous solution of the crystallized product was acidic (pH 4.5) indicating the free phenolic hydroxyl. The identity of the product was established by melting point, colour reactions and the preparation of methyl ether, lactone, and acetyl derivatives.

TABLE I.

S. No.	Batch No.	Potency of first crystals of potassium penicillin G. u/mg.
1	E 905/909	820
2	E 812/813/814—II Part	509
3	E 812/813/814—I Part	1100
4	E 533-34-35	890

The strain of *P. chrysogenum* in use during the period of study was H. A. 3, a derivative of Wis. 51-20, in peanut meal, cornsteep lactose medium. Out of a large number of batches studied during this period only in six batches the abnormal fermentations were noticed. No difference in fermentation conditions could, however, be traced in these batches. In all the abnormal cases the penicillin titres were only 50-60 per cent of the normal titres.

The isolation of *o*-hydroxyphenylacetic acid in such large quantities lends support to the postulate of oxidative metabolism of the precursor. The occurrence in two batches of this oxidative product to the extent of almost 50 per cent of the amount of the precursor was very abnormal. However, in a systematic investigation of other batches using the same strain under identical fermentation conditions the presence of *o*-hydroxyphenylacetic acid was not detected either in the crude potassium penicillin G or in the mother liquor.

Experimental

Isolation of o-hydroxyphenylacetic acid

The rich aqueous extract of potassium penicillin, obtained by extracting the rich organic solvent extract (butyl acetate extract) with aqueous potassium acetate, was treated with saturated potassium acetate solution. The crude crystals of potassium penicillin were centrifuged, washed with butanol and acetone. Potency of penicillin varied from 600 u/mg. to 1100 u/mg. in the different batches. The *o*-hydroxyphenylacetic acid was separated from this mixture by two methods :

(1) The crude crystals (1 kg., potency 820 u/mg.) were suspended in 2 l. of ice-cold water, stirred and filtered. The residue was then washed with ice-cold water (2 x 500 ml.) to remove all the penicillin. On recrystallizing the residue from hot water, the potassium salt crystallized as glistening white flakes, m.p. 245-55°. Aqueous solution of the sample had pH 4.5. The mono-potassium salt was acidified with hydrochloric acid and extracted with ethylacetate. Distilling off the ethylacetate and crystallizing the residue gave fine prismatic crystals (I), m.p. 147-48°, mol. wt. 154 (calc. 152). Crude crystals melted at 139-41°, yield 250 g. (I) gave violet colour with ferric chloride in aqueous solution, and was readily soluble in aqueous sodium bicarbonate with effervescence. Properties of (I) agreed with those of

o-hydroxyphenylacetic acid, and the identity was confirmed by preparing the following derivatives : Methyl ether, m.p. 121-23°, did not show any depression in melting point when mixed with authentic sample obtained from salicylaldehyde methyl ether by the azalactone method[6]; lactone, m.p. 47-49°, b.p. 248-50[7] ; with acetic anhydride and pyridine (I) gave an acetyl derivative, which crystallized from alcohol as orange red needles, m.p. 118-20°, agreeing in its properties with the C-acetyl derivative of isocoumaranone described by Chatterjea.[8]

(2) The low potency first crystals obtained from the production batches, were dissolved in water to a concentration of 150,000 u/ml. of penicillin. The penicillin was precipitated as the procaine salt by addition of procaine hydrochloride in slight excess, filtered, and washed with water. The filtrate was acidified to pH 2 with dilute hydrochloric acid and left for 2 hours to decompose all the penicilin. The filtrate was then twice extracted into butylacetate, and the acetate layer washed with water. On extracting the butylacetate extract with aqueous bicarbonate and acidifying the aqueous extract, crystalline material separated out. Recrystallization from chloroform gave pale coloured needles, m.p. 147-49°, identical with (I).

Alternatively, the butylacetate layer was extracted with aqueous potassium acetate (30 per cent w/v) and the aqueous extract treated with an equal volume of saturated potassium acetate solution. The potassium salt that crystallized out was filtered and washed with ice-cold water. Recrystallizing from hot water yielded glistening white flakes, m.p. 245-50°. An aqueous solution of the sample had a pH 4.5. Its properties agreed with those of a mono-potassium salt of *o*-hydroxyphenylacetic acid described above.

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Control of Black Rot of Cabbage with Citrinin

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BLACK rot of cabbage incited by *Xanthomonas campestris* (E. F. S.) Dowson is an internally seed-borne disease distributed in many parts of the world. Considerable damage is caused to cabbage and cauliflower plants by stunting of growth, deformed heads, etc. In the United States of America where considerable work has been done, the disease is controlled by growing certified disease-free seeds obtained from Puget Sound area, and by using seed treatments such as with mercuric chloride solution and hot water. While the incidence of the disease is effectively minimized by these treatments, the germination of the seeds is considerably reduced. In the case of hot water treatment, the seeds are soaked for 25 min. in water at 122°F. The treatment is almost on the borderline of killing the seeds also.

The possibilities of using antibiotics in controlling plant diseases have been investigated in recent years. In spite of the enormous cost involved, antibiotics have proved beneficial in many cases where conventional types of fungicides and bactericides have failed.

While there are numerous reports on the use of antibiotics as foliar and blossom sprays, dip and seed treatments against fungal and bacterial diseases, the dip and seed treatments hold out promise in India as they are economical and do not involve large quantities of the antibiotics. As regards control of black rot of cabbage, Sutton and Bell[1] have reported that a 30-minute immersion of seed in 1:1,000 chlortetracycline hydrochloride solution followed by drying for at least three days gave control of black rot of swedes. In the present work, the possible use of citrinin as seed treatment agent for cabbage in relation to disease control and phytotoxicity was investigated, and a brief account is presented here.

Unlike other antibiotics which need elaborate processes for manufacture, citrinin can be easily produced under laboratory conditions. A strain of *Aspergillus candidus* Thom. was inoculated in Erlenmeyer flasks containing Czapek-Dox solution and incubated at room temperature (24-28°) for 20 days. After 20 days of surface culture, the broth was filtered and acidified to pH 2 with hydrochloric acid. Crude yellow crystals of citrinin separating out were filtered and recrystallized. For testing antibacterial activity, the crystals were dissolved in a solution containing sodium citrate 1.05 per cent and sodium chloride 0.8 per cent. Comparison with an authentic pure sample of citrinin indicated that the sample prepared in the laboratory was 95 per cent pure. All the studies on control of black rot of cabbage were carried out with the citrinin sample prepared in the laboratory.

Freshly isolated cultures of *X. campestris* from black rot affected cabbage plants were used for finding out the concentration of citrinin required for complete inhibition *in vitro*. In turbidimetric assay 1 μg/ml. of citrinin inhibited all strains of *X. campestris* that were tested.

Effect of seed treatment in controlling the disease was studied in the green house. Seed boxes containing sterile soil (first sterilized by 1:120 solution of formalin and later by steam) were planted with treated seeds and observations made for germination and disease incidence. Each lot consisting of 1,000 seeds were treated by soaking them for 2 hr. in different concentrations of citrinin, and 600 of the treated seeds were planted (3 replicates of 200 seeds) and kept for observation. Several controls were set apart in each case. The diseased seedlings showed characteristic bacterial lesions on the cotyledons and blackening of the veins. The bacterial ooze could be easily seen under the microscope. The results of treatments are given in Table I.

TABLE I.

EFFECT OF SEED TREATMENT WITH DIFFERENT CONCENTRATIONS OF CITRININ ON SEED GERMINATION AND BLACK ROT INCIDENCE

(200 seeds planted in each set)

Citrinin conc. (μg/ml.)	Seed germination %	Diseased seedlings %	Healthy seedlings %
0.0 ..	85	90	10
1.0 ..	85	70	30
5.0 ..	76	20	80
50.0 ..	66	10	90
100.0 ..	60	8	92
500.0 ..	50	8	92

The data indicate that different concentrations between 5 μg/ml. and 50 μg/ml. could be effective in reducing the disease incidence and also be fairly non-phytotoxic. However, in actual field practice seed treatment with 50 μg/ml. concentration of citrinin and 50 per cent more seed rate to make up the loss of reduced germination gave excellent nursery beds with healthy seedlings. The 90 per cent disease incidence noticed in the controls was chiefly due to heavy inoculum carried within the seed since they were collected from severely diseased plants. The quantity of citrinin required being quite small, it holds promise of being useful in seed treatment in the control of black rot of cabbage.

ACKNOWLEDGEMENT

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Preservation of Ready Inoculum of *Penicillium chrysogenum* Spores for Penicillin Fermentation

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MAINTENANCE of productivity of industrially important strains of micro-organism is of primary importance in fermentation plants. Soil storage, lyophilization, refrigeration, etc., are the common methods for preservation of master cultures[1].

Spore inoculum for seed preparations is generally made fresh from the master cultures or subcultures thereof for each batch of fermentation. Although the problem of selecting the best methods of preserving master cultures continues to be investigated, there appears to be no published report on the maintenance of productivity of a given preparation of mass culture of spores suspended in a suitable vehicle ready for transfer to seed tanks.

The purpose of the present study was to investigate the possibility of preserving penicillin productivity of a ready inoculum of a spore suspension of *P. chrysogenum* for a substantially long period with a view to eliminate the variables in operations in the industry involving preparations of spore inoculum from master tubes (usually takes 8-10 days for mass sporulation).

Material and Methods

A commercial strain of *P. chrysogenum* (HA-6), a selection from a Russian stock was used for the study.

Spores from an agar slant suspended in honey-peptone solution (about 20 ml.) were aseptically mixed with previously sterilized barley grains (100 g.) in a 500 ml. Erlenmeyer flask and incubated at 25°. After 8 days of incubation the spores formed on barley grains were collected by shaking the grains with sterile distilled water (200 ml.) Approximately 40 billion spores were obtained as water suspension.

About 5 billion spores were transferred to each of seven tubes and the supernatant water poured off after the spores settled at the bottom. The tubes were serially numbered 1 to 7 and the spores in the respective tubes treated as follows :

Tube 1 — Mixed with sterile garden soil (10 g.)

Tube 2 — Suspended in sterile water (10 ml.)

Tubes 3, 4 and 5 — Suspended in sterile water (10 ml.) containing respectively sodium azide (1 mg.), KCN (0.1 mg.), and parachloromercuribenzoic acid (PCMB) (0.1 mg.) as metabolic inhibitors.

Tube 6 — Suspended in 50 per cent glycerol (10 ml.)

Tube 7 — Suspended in glycerol (10 ml.)

All the tubes were stored in the refrigerator (5°) and the penicillin productivity of spore suspensions in each of the tubes 2 to 7 determined and compared with that of the soil stock culture (tube 1) at intervals of about 4 months.

Fermentation Method

Spore inoculum : Spore suspensions (0.1 ml.) from tubes 2 to 7 and soil (about 0.1 g.) from tube 1 suspended in water (2 ml.) were directly inoculated into seed medium (100 ml.) containing cornsteep liquor 3 per cent, sucrose 2 per cent and salts. Forty-eight hr. vegetative inoculum (10 ml.) was transferred to fermentation medium (100 ml.) containing cornsteep liquor 1 per cent, peanut meal 3 per cent, lactose 4 per cent, phenylacetic acid 0.1 per cent and salts. All fermentations were carried out in duplicates at 25° on a rotary shaker (240 r.p.m., 2″ throw). Penicillin was estimated at 120 hr. of fermentation by the modified iodometric method[2].

Results and Discussion

Maintenance of penicillin productivity of the different spore preparations preserved for a period of 20 months are given in Table I. Under the given fermentation conditions the starting stock culture showed an average titre of about 2,200 u/ml. This productivity was preserved undiminished for at least 12 months in 100 per cent glycerol suspension. In plain water suspension also the spores could be preserved for at least 4 months at 5° without appreciable deterioration in productivity. Comparatively low titre value in all cases after 8 months storage (Table I column 4), was traced at a later stage to be due to an inferior quality of cornsteep liquor used for the particular set of fermentation.

TABLE I.

PENICILLIN PRODUCTIVITY IN READY INOCULA OF *P. chrysogenum* SPORES STORED AT 5°.

Tube No.	Media	Period of Storage (months)				
		4	8	12	16	20*
		u/ml.	u/ml.	u/ml.	u/ml.	u/ml.
1.	Soil	2275	1850	1875	1770	1775
2.	H_2O	2000	1675	—	—	—
3.	Azide ..	2150	1750	—	—	—
4.	KCN ..	2175	1825	—	—	—
5.	PCMB ..	2275	1825	—	—	—
6.	Glycerol(50%)	2350	1850	1625	1530	—
7.	Glycerol ..	2225	1850	2220	1925	1500

* Penicillin titres at 144 hr. All other data are for 120 hr. of fermentation.

Metabolic inhibitors at the levels added did not seem to check deterioration of productivity in aqueous suspensions of spores. Harmful effect of water was also apparent when spores were suspended in aqueous glycerol (50 per cent).

From the performance of the soil stock it appears that the soil culture started deteriorating after 12 months. The deterioration was much more than that in glycerol suspension (1,770 u/ml. as compared to 1,925 u/ml. after 16 months' storage). The spores in aqueous suspensions with or without metabolic inhibitors when used as inoculum after 12 months' storage produced very little mycelial growth in seed medium upto 48 hr. presumably due to loss in viability. Pellety growth was obtained by prolonging the seed stage upto 96 hr. Penicillin productivity of these seed mycelia was not determined, and further studies with these spore preparations were discontinued.

With spores preserved in glycerol for 20 months, 0.1 ml. suspension did not grow in seed medium. Growth was, however, normal when 1.0 ml. of suspension was employed as inoculum. Final penicillin titre assayed at 144 hr. was appreciably low, 1,500 u/ml. as against 1,925 u/ml. after 16 months' storage. This indicated about 90 per cent loss in viability in glycerol suspension after 20 months' storage.

Deterioration in glycerol was very fast after 20 months as was evidenced by the fact that even with 1.0 ml. of suspension there was no growth in seed medium when tested a month later. However, when slants made out of the glycerol tube and soil stock were used as spore inocula the productivity of both the preparations was nearly the same (Table II). This accelerat-

TABLE II.

PENICILLIN PRODUCTIVITY OF SLANTS FROM SOIL STOCK AND GLYCEROL SUSPENSION AFTER 21 MONTHS STORAGE

Inoculum	Hours of fermentation					
	96		120		140	
	u/ml.	pH	u/ml.	pH	u/ml.	pH
Soil slant	1440	7.7	1930	7.9	1700	7.95
Glycerol slant ..	1220	7.5	1800	7.9	1900	7.9
Glycerol stock (1 ml.)	No growth in seed medium					

ed deterioration in glycerol after 20-months storage might have been due to the presence of considerable amount of moisture in the original spore stock which was prepared simply by allowing the aqueous suspension to settle and decanting off the supernatant water. With hard centrifugation and washing with glycerol the productivity might be preserved undiminished for a much longer period.

Beneficial effect of glycerol in preserving cell structure and biochemical characters was first reported by Polge *et al*[3]. They observed that the motility of certain mammalian and avian spermatozoa was preserved in presence of glycerol even after freezing and thawing. The use of glycerol for preservation of living cells at low temperature was discussed by Smith *et al.*[4] Hollander and Bell[5] and more recently Howard[6] observed improved preservation of cell structure and viability of bacteria by freezing with glycerol.

The present work with spores of *P. chrysogenum* clearly indicates that in industrial penicillin fermentation glycerol suspensions of spores once made by mass sporulation in large number of barley flasks could be preserved as ready inocula and used routinely for a period of at least one year with undiminished productivity. The advantage of this practice in respect of maintenance of productivity and economy in routine mycological operations in antibiotic plant laboratories is obvious.

It would be worthwhile to do a long term study of maintenance of spore viability in frozen glycerol suspension in comparison with the classical methods of preservation of master cultures. Similar studies with glycerol could also be made with other microorganisms of industrial importance.

ACKNOWLEDGEMENT

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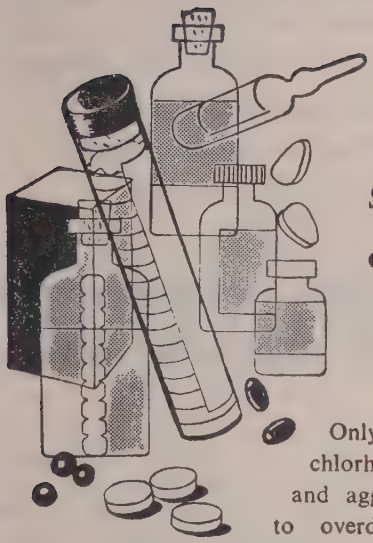

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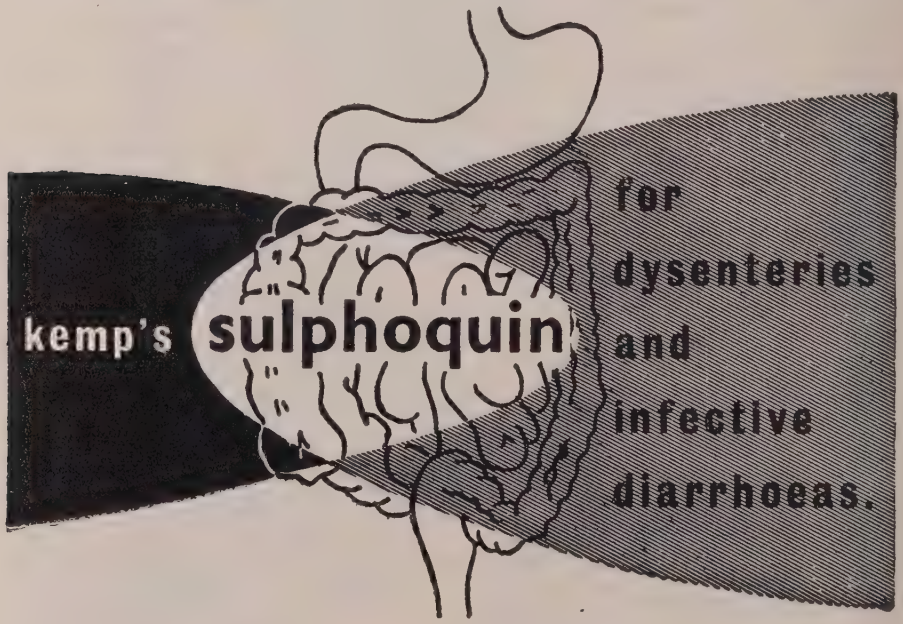

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Physico-chemical Data on Antibiotics

II.[1] ANTIBIOTICS PRODUCED BY *ACTINOMYCETES*

1. Antibiotics with antifungal activity.

SYSTEMATIC screening of soil samples to detect microorganisms, with antibiotic activity was more specifically directed to screening of *Actinomycetes* following the isolation of streptothricin (1941-42) and streptomycin (1945) from cultures of *Streptomyces* spp. The broad-spectrum antibiotics—chloramphenicol (1947) and the tetracyclines (1948-53)—resulted from such planned programmes.

Production of an antifungal antibiotic (cycloheximide) by *S. griseus* was reported in 1946 ; the discovery of thiolutin, endomycins, and nystatin followed almost consecutively in 1950-51. By the end of 1959 isolation of over 500 antibiotics from *Actinomycetes* has been reported in literature. Of these a little over 150 compounds are active against fungi, and their physical-chemical characteristics are recorded in the present compilation.

The detection of a large number of antifungal substances, particularly polyenes, in *Streptomyces* cultures has been reported in literature, but only some 60 of the polyenes have been isolated and studied in any detail.

Some of the antifungal compounds are also active against other types of microorganisms ; such additional activity is indicated in column 5 of the table.

In the table the antibiotics are grouped as follows :

POLYENES[2] (S. No. 1-57a)

Tetraenes, pentaenes, hexaenes, heptaenes.

NON-POLYENES

Compounds containing C, H, O only (S. No. 58-72).

(1) By empirical formulae,[3]
(2) other compounds, empirical formula not known.

Compounds containing C, H, N, O (S. No. 73-125).

(1) By empirical formulae,[3]
(2) other compounds, empirical formula not known.

Compounds containing, C, H, N, O, S (S. No. 126-132).

(1) By empirical formulae,[3]
(2) other compounds, empirical formula not known.

MISCELLANEOUS COMPOUNDS (S. No. 133-152).

Literature references are given in column 5. Whenever an antibiotic is described in Spector's Handbook,[4] reference is made to it as *H. T.* 2. *C. A.*=*Chemical Abstracts.*

An asterisk (*) following a S. No. indicates that the full or partial structure of the antibiotic is known.

As for previous data tables, indexes to the melting point, ultraviolet absorption maxima, and empirical formulae are provided in charts 1, 2 and 3 respectively. The melting point range extends from 70° to 230° C in steps of 5°, and the ultraviolet maxima from 210 to 550 mμ by unit intervals. Chart 3 indexing empirical formulae can be used in the same way as the index for ultraviolet data. For example, antibiotics with C_{12} in the table are S. Nos. 76, 77, and 78, those with H_8 are S. Nos. 58, 73, 76 and 128, and so on ; $C_{12}H_8$ would be for antibiotic at S. No. 76.

A. NEELAMEGHAN.
Library,
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1 Part I, (1)-(3), *Hindustan Antibiot. Bull.* **2**, 13, 54, 113 (1959-60)

2 Classified by ultraviolet absorption maxima (Cf. *J. Gen. Microbiol.* **17**, 97 (1957))

3 Closely related compounds are grouped together even when empirical formula is not known.

4 Spector, W. S., *ed.* Handbook of toxicology. Saunders, 1957. V. 2.

ANTIFUNGAL ANTIBIOTICS PRODUCED BY *ACTINOMYCETES*

S. No.	Antibiotic & Producing organism.	Chemical nature. Melting point (°C)	U. V. absorption maxima (λ max) mμ. Optical rotation (α)$_D$	Remarks. References.
1	2	3	4	5
		POLYENES		
1*	PIMARICIN *S. natalensis*	Yellow cryst.; colourless pure compound. Amphoteric. Conjugated tetraene. Macrolide. $C_{34}H_{49}NO_{14}$ Dec. approx. 200 without definite m.p.	222, 279, 290, 303, 318	*Antibiot. Ann.* **1957-58,** 878. *J. Am. Chem. Soc.* **80,** 6688, 6689, (1958). *C.A.* **53,** 11750b (1959). *Antibiot. and Chemother.* **8,** 381 (1958)
2	PA 166 *Streptomyces* sp.	Colourless. Amphoteric. Conjugated tetraene. $C_{35}H_{53}NO_{14}$	319, 304, 291 (80% aq. MeOH) +275 (pyridine, 25°), +257 (dimethyl formamide, 25°), +191 (dimethyl-formamide-0.1N HCl, 25°)	*Antibiot. Ann.* **1957-58,** 893, 897.
3	ETRUSCOMYCIN *S. lucensis*	Unsaturated tetraene. $C_{36}H_{57}NO_{14}$	304-305 +50 (0.1N HCl, 20°), +296 (pyridine, 20°)	*Ann. Chim.* (*Rome*) **49,** 345 (1959)
4	NYSTATIN *S. noursei*	Needles (from MeOH), yellow powder. Amphoteric. Tetraene. $C_{46}H_{77}NO_{19}$. Gradual dec. above 160 without melting by 250.	230, 292, 305, 320; 280, 291, 304, 318.	*H. T.* 2, *Trans. N. Y. Acad. Sci.* **19,** 447 (1957). *Inter. Cong. Biochem.* 3, *Brussels Res. Com.*, 1955, 1.
5	ANTIMYCOIN A *S. aureus*	Tetraene. Similar to (Nystatin (4). Acid labile. Alkali stable, thermostable pH 7	290, 305, 318, 320	*Antibiot. and Chemother.* **2,** 179 (1952). *Antibiot. Ann.* **1957-58,** 869.
6	ANTIBIOTIC *S. fungicidicus*	Similar to Nystatin (4). Positive Fehling, Molisch. Negative Millon, Sakaguchi, Schiff, Tollen, $FeCl_3$. Blue with $FeCl_3$-K ferricyanate; decolourizes $KMnO_4$.	290, 303, 317	*C. A.* **52,** 9530f (1958)
7	ANTIBIOTIC *Streptomyces* sp.	Nystatin type. Second substance produced in broth. Amphoteric. Tetraene.	290, 305, 316. Second substance has no U. V. max.	*C. A.* **52,** 13859h (1958)
8	RIMOCIDIN *S. rimosus*	Sulphate: Large, fragile plates. Amphoteric. Tetraene. C 57.65, H 7.82, N 1.81, S 2.03. Sulphate: 151 dec.	279, 291, 304, 318 (MeOH) Sulphate: +75.2 (c, 1 MeOH, 25°)	Also active against protozoa. *H. T.* 2

1	2	3	4	5
9	AMPHOTERICIN A *Streptomyces* sp.	Amphoteric. Conjugated tetraene. C 60.32, H 8.39, N 1.72. Positive Molisch; decolourizes $KMnO_4$ or Br_2-CCl_4. Negative $FeCl_3$. Stable pH 6 and 7 at 30°.	291, 305, 320 (70% propanol or MeOH)—9.9(0.1N methylhydroxide-HCl, 23.5°); +32 (acid dimethylformamide, 23.5°)	*H. T.* 2
10	SISTOMYCOSIN *S. viridosporus*	Buff or light yellow microcryst. solid. Neutral. Tetraene. Positive Molisch; reduces $KMnO_4$, boiling Benedict's soln. Negative Beilstein, $FeCl_3$. Turns brown 130, melts approx. 230.	218, 292.5, 306, 320.5.	*H. T.* 2
11	ENDOMYCINS *S. endus*, and species related to *S. albus*.	Crude mixture yellowish brown powder. Acidic. Endomycin A is tetraene, B (q.v.) is hexaene. Thermostable at pH 7-10	Mixture: 226, 232. A: 290, 305, 325.	Also active against gram+ bacteria, some protozoa. *H. T.* 2. *Canad. J. Chem.* **35**, 1461 (1957.)
12	AKITAMYCIN *S. akitaensis*	Tetraene. C 57.26, H 7.68, N 1.64.	291, 303.5, 319. +158 (0.5% dimethylformamide, 25°); +88 (80% MeOH, pH 8.8, 23°)	*J. Antibiot.* (*Jap.*) **12B**, 293, 295, 297 (1959)
13	YUNAMYCIN *S. fungicidicus*	Tetraene. Negative $FeCl_3$, Millon, Schiff, Sakaguchi, Tollens. Decolourizes Br_2-CCl_4, $KMnO_4$; reduces Fehling. Dark green and blue with conc. CHl and $FeCl_3$-K_4 ($Fe(CN)_6$) soln. resp.	290, 304, 320 (50γ/cc in MeOH)	*C. A.* **53**, 20708d (1959)
14	TENNECETIN *S. chattanoogensis*	Yellow amorphous powder. Tetraene. Aq. soln. alkaline. Soln. stable, insensitive to light; stable pH 7. Stability decreases pH 4 and 10.	288, 300-302, 315-318, sh. at 270-280.	*Antibiot. and Chemother.* **9**, 398, 406 (1959).
15	PROTOCIDINE *Streptomyces* sp. (Mycelium)	Crystalline. Negative biuret, Sakaguchi, Molisch, ninhydrin, anthrone, $FeCl_3$. Reduces $KMnO_4$; Fehling green colour. Changes colour to brown about 120°, but no clear m.p.	277, 290, 303, 318 (80% MeOH)	Also active against protozoa. *J. Antibiot.* (*Jap.*) **10A**, 128 (1957)
16	CHROMIN *Streptomyces* sp., resembling *S. antibioticus*.	Fine white needles. Tetraene. C 58.19, H 7.81, N 2.29. Reduces Fehling. Negative ninhydrin, Molisch, biuret, $FeCl_3$, Sakaguchi, Millon. Thermo, acid, alkali labile.	281, 292.5, 305, 320; 289, 303, 317.	*H. T. 2.*

1	2	3	4	5
17	ANTIBIOTIC *Streptomyces* sp., resembling *S. fungicidicus*	Powder. Resembles Chromin (16). Sakaguchi positive.		*C. A.* **51**, 18101b (1957)
18	A 284, A 288, A 387, A 432, A 862. *Streptomyces* spp.	Tetraenes.	290, 305, 318.	*J. Gen. Microbiol.* **17**, 96 (1957)
*18a	FLAVOFUNGIN *S. flavofungini*	Yellowish green, fine needles. $C_{30}H_{48}O_9$. Unstable to light and air. Contains 7 OH groups, 5 C=C double bonds of which at least 4 are conjugated.	263, 368.	*Nature* **181**, 908 (1958). *Arzn. Forsch.* **9**, 178 (1959). *Angew Chem.* **72**, 139 (1960).
19	PA 153 *Streptomyces* sp.	Amphoteric. Conjugated pentaene. Colourless soln. shows strong grayish green fluorescence in U. V. Positive ninhydrin, 2, 4-dinitrophenylhydrazine, Fehling. Violet with conc. H_2SO_4. $C_{37}H_{61}NO_{14}$.	303, 317, 332, 349 (80% aq. (MeOH) +398 (pyridine, 25°); +296 (dimethylformamide, 25°); +353 (dimethylformamide-0.1N HCl)	*Antibiot. Ann.* **1957-58**, 893, 897.
*20	LAGOSIN (A 246) *Streptomyces* sp.	Pentaene. Macrocyclic lactone. $C_{41}H_{78\cdot80}O_{14}$. 235 dec.	318, 333, 351.—160 (c, 0.2 MeOH, 20°)	*J. Gen. Microbiol.* **17**, 96 (1957), *Proc. Chem. Soc.* (1958) 148; *ibid.*, (1959), 154.
21	ALIOMYCIN *S. acidomyceticus*	Yellowish brown powder. Pentaene. Positive Fehling (on heating); weakly positive Molisch, Seliwanoff; red purple with conc. H_2SO_4. Contains C, H, N, O, S.	321, 330, 351	Also active against Yoshida sarcoma *J. Antibiot.* (*Jap.*) **9B**, 101 (1956)
22	EUROCIDIN *S. albireticuli*	Pentaene.	318, 332, 350	*H.T.* 2. *C.A.* **52**, 9529h, (1958)
23	ANTIBIOTIC *S. effluvius*	Pentaene.	305, 317, 333, 350.	Also active against some protozoa. Ger. Pat. 1,012,430 (1958)
24	ANTIBIOTICS *S. griseus*	Pentaenes.	320, 333, 350.	*Antibiot. Ann.* **1955-56**, 251.
25	A 228, A 341, A 786, A 789 *Streptomyces* spp.	Pentaenes.	318, 333, 351.	*J. Gen. Microbiol.* **17**, 96 (1957)
*26	FILIPIN *S. filipinensis*	Fine feathery needles. Neutral. Conjugated pentaene. $C_{32}H_{54}O_{10}$. 195-205	322, 338, 355, sh. 305 —148·3 (c, 0·89 MeOH, 22°)	*H.T.* 2. *Proc. Chem. Soc.* (1959), 316. *C.A.* **52**, 3277b (1958)

1	2	3	4	5
*27	FUNGICHROMIN *S. cellulosae*	Pale yellow cryst. Conjugated pentaene. Violet changing to blue with conc. H_2SO_4; Tollens reduced slowly; decolourizes Br, $KMnO_4$. $C_{35}H_{60}O_{13}$. 205-210 uncor.	311, 323·5, 339, 357	*H.T.* 2. *J. Am. Chem. Soc.* **80**, 1504 (1958)
28	FUNGICHROMATIN *Streptomyces* sp.	Similar to Fungichromin (27)	318, 333, 351, sh. 305.	*Antibiot. Ann.* **1954-55**, 716
29	MOLDCIDINE A *Streptomyces* sp.	Pentaene. Positive ninhydrin. Negative biuret, Fehling, Molisch, Sakaguchi, $FeCl_3$. C. 55. 69, H 8. 93, N 1.48. 180-230 slowly with frothing	324, 339, 358 (80% MeOH)	*J. Antibiot.* (*Jap.*) **12A**, 169 (1959)
30	A 772 *Streptomyces* sp.	Pentaene.	325, 340, 358	*J. Gen. Microbiol.* **17**, 96 (1957)
31	ANTIBIOTIC *Streptomyces* sp. (Mycelium)	White powder. Pentaene, with some resemblances to Eurocidin (22).	322·5, 338.5, 356·5, sh. 310.	*J. Antibiot.* (*Jap.*) **9A**, 125 (1956).
32*	PENTAMYCIN *S. penticus* (Mycelium)	Slightly yellow cryst. Pentaene, the chromophore 2-methyl-2, 4,6, 8, 10-dodecapentaenedial (Same as chromophore of Fungichromin) (27). Deep purple with conc. H_2SO_4, reduces Tollen slowly; decolourizes Br-water, negative Fehling, $FeCl_3$. C, H, O only. 236-37 dec., 242-242·5 dec.	322, 338, 356, sh. 308-313	*J. Antibiot.* (*Jap.*) **11A**, 26, 273 (1958) *C.A.* **53**, 22764e (1959)
33	ANTIBIOTIC *S. roseoflavus*, *S. fradiae*, *S. diastatochromogenes*	Dark yellowish powder (crude) Hexane. Fradicin-Mycelin type.	243, 294, 335, 355, 373.	Also active against protozoa. *J. Antibiot.* (*Jap.*) **12A**, 73 (1959)
34	FRADICIN *S. fradiae*	Light greenish yellow. Weak base. Hexaene. $C_{30}H_{34}N_1O_4$. 180-300 darkens without melting.	340, 356, 378. +65 (c, 1.01, 1, 4-dioxane, 25°)	Also active against protozoa. *H.T.* 2
35	MYCELIN *S. roseoflavus*	Prisms (from acetone). Hexaene. Negative Molisch. No N or S. 260 blackens, 263 dec., uncor.		*H.T.* 2

1	2	3	4	5
36	MYCELIN-IMO *S. diastatochromogenes*	Yellow cryst. Dark green colour with conc. H_2SO_4. Hexaene. Thermostable. 214-22 dec.	243, 294, 335, 355, 373. +70 ±2 (c, 1% in 1, 4-dioxane, 21°)	*C.A.* **52**, 20917h (1958)
37	SUBSTANCE 207 *S. fradiae*	Yellow plates. Hexaene, Fradicin-Mycelin type. C 70·88, H 6·05, N 11·31. 210-22 dec.	As for Fradicin (34)	*C.A.* **53**, 18180g (1959)
38	SUBSTANCE 1404 *S. diastatochromogenes* (Mycelium)	Yellow cryst. Hexaene, Fradicin-Mycelin type. N 10·47; no S, halogen. 210-20 dec.	Same as for Fradicin. +67·5 ±2 (c, 1 dioxane, 21°)	*CA.* **53**, 18170g (1959)
39	MEDIOCIDIN *S. mediocidicus*	Yellow powder. Hexaene.	305-306, 320-322, 339-340, 356-357. 377-378.	*H.T.* 2 Slight activity against Ehrlich carcinoma.
40	FLAVACID *Streptomyces* sp. resembling *S. flavus*	Yellow cryst. powder. Hexaene. Acidic, isoelectric point pH 5·8-6·6. Yellow at neutral pH, green at acid, red at alkaline. Ppt. with Ba, Ca, Pb, Zn. Negative ninhydrin, $FeCl_3$. 102-105 dec.	340, 360, 380; 341, 358, 379.	Also active against a few bacteria and *T. vaginalis*. *H.T.* 2.
41	ENDOMYCIN B' *S. endus*, *Steptomyces* sp. related to *S. albus*	(Cf. 11). Hexaene.	340, 352, 380	Endomycins also active against gram + bacteria, some protozoa. *H.T.* 2. *Canad. J. Chem.* **35**, 1461 (1957)
42	CRYPTOCIDINE *Streptomyces* sp.	Heptaene. Negative biuret, Sakaguchi, Molisch, Fehling, ninhydrin, $FeCl_3$. Deep blue with conc. H_2SO_4. No S or halogen. 110-15 with frothing.	290, 305, 320, 341, 358, 380 (80% MeOH)	Also active against protozoa. *J. Antibiot.* (*Jap.*) **12A**, 21 (1959)
43	CANDIDINS *S. viridoflavus*	Heptaenes. Ppt. with dil. acetic or mineral acids. Negative Molisch, Benedict, Fehling, Tollens, Schiff, pine splint, $FeCl_3$; deep blue with H_2SO_4; decolourize Br-$CHCl_3$ and $KMnO_4$. Positive for ketones. Contain N, but negative ninhydrin after hydrolysis.	Na salt: 234, 282, 345, 360, 383, 405	*H.T.* 2

1	2	3	4	5
44	CANDICIDINS A, B, C. *S. griseus*, *Streptomyces* spp.	Heptaenes. Crude compound non-dialyzable.	A: 360, 380, 403. B: 362, 381, 404. C: 358, 379, 402.	*H.T.* 2 C relatively inactive.
45	TRICHOMYCINS A, B, C. *S. hachijoensis*	Yellow amorphous powder. Heptaenes. Negative $FeCl_3$, ninhydrin, biuret, Fehling, Benedict, Molisch, Tollens, quinone reactions. Ppt. with metallic salts, dyes, enzymes, acridine deriv. Blue colour changing violet with H_2SO_4 or HCl. Contains C, H, O. 155 dec.	286, 346, 364, 384, 405.	Also active against spirochaetes, protozoa. *H.T.* 2.
46	ANTIBIOTIC *Streptomyces* sp. (producing Phleomycin)	Yellow powder. Heptaene. Trichomycin-like.	360-61, 380-81, 403	*J. Antibiot.* (*Jap.*) **9A**, 82 (1956)
47	ANTIBIOTICS *Streptomyces* spp.	Heptaene. Probably contain unsaturated conjugated double bond. One antibiotic (No. 757) closely related to Ascosin (55).	360, 380, 400 (MeOH)	Also antimytotic effects on *Allium cepa* root meristem. *C.A.* **51**. 18157a (1957)
48	ANTIBIOTICS *S. griseus*	Heptaenes.	359-362, 378-382, 401-405.	*Antibiot. Ann.* **1955-56**, 251.
48a	A 4, A 571, A 583 *Streptomyces* spp.	Heptaenes.	360-378, 405.	*J. Gen. Microbiol.* **17**, 96 (1957)
49	ANTIBIOTIC *Streptomyces* sp.	Yellow amorphous powder. Precipitated from NaOH by various salts.	361, 381, 404	Also affects cell multiplication. *C.A.* **15**, 1499h (1957)
50	ANTIBIOTIC *S. abikoensis*	Yellow powder. Heptaene, Trichomycin-like; Actinoleukin in mycelium.	242, 358, 400 (EtOH)	Also active against some gram+bacteria. *J. Antibiot.* (*Jap.*) **9A**, 86, (1956)
51	PA 150 *Streptomyces* sp.	Yellow substance. Amphoteric. Conjugated heptaene. Positive Fehling, 2, 4-dinitrophenyl-hydrazine; blue with conc. H_2SO_4. $C_{54}H_{82}N_2O_{18}$.	397, 377, 358, 340 (80% aq. MeOH) +29 (pyridine, 25°); + 148 (dimethylformamide, 25°); —34 (dimethylformide-O.1N HCl, 25°)	*Antibiot. Ann.* **1957-58**, 893, 897.

1	2	3	4	5
52	AMPHOTERICIN B *Streptomyces* sp.	Amphoteric. Conjugated heptaene. Positive Molisch, decolourizes $KMnO_4$ or Br_2-CCl_4; negative $FeCl_3$. Does not form MeOH-sol. $CaCl_2$ complex. $C_{46}H_{73}NO_{20}$. Gradual dec. over 170	364, 383, 408 (70% propanol); 363, 382, 406 (MeOH). —33·6 (O.1N methanolic HCl, 23·5°); +333 (acid dimethylformamide, 23.5°)	*H.T.* 2. *Antibiot. Ann.* **1956-57**, 866
53	ANTIBIOTIC 1968 *S. aminophilus*	Heptaene. U. V. absorption at identical wavelengths as for Ascosin (55) but at different densities.	As for Ascosin (55)	*Antibiot. Ann.* **1955-56**, 236, 240
54	AYF-A, B. *S. aureofaciens*	Dark yellow cryst. ppt. Weakly acidic heptaenes. Analysis for A and B average: C 62·5, H 7.8, N 2.8.	344, 363, 383, 403 (dimethylacetate)	*Antibiot and Chemother.* **8**, 491 (1958)
55	ASCOSIN *S. caneseus*	Heptaene. Negative Molisch, Tollens, Ehrlich, $FeCl_3$, ninhydrin, Benedict, Sakaguchi.	234, 288, 348, 357, 376, 398 (MeOH)	*H.T.* 2.
56	CANDIMYCIN *S. chimensis*	Yellow powder. Heptaene. Negative ninhydrin, $FeCl_3$, Molisch, Fehling, Sakaguchi. Postitive Ag mirror; blue then violet with H_2SO_4. Darkens at 150, no melting by 230.	362, 382, 406 (MeOH)	*C.A.* **51**, 18494a (1957)
57	ANTIBIOTIC 26/1 *A. globisporus*	Heptaene. Alc. sol. turns violet with H_2SO_4; decolourizes $KMnO_4$. Negative biuret, xanthoproteic, ninhydrin.	359, 380, 404 (EtOH)	*Antibiotiki* **4**, No. 1, 21 (1959)
57a	EUROTIN A *Streptomyces* sp.	Heptaene. Contains C, H, N, O.	360, 380, 405	*J. Antibiot.* (*Jap.*) **12B**, 368 (1959)

NON-POLYENE SUBSTANCES

Compounds Containing C, H, O, only

58	SARKOMYCIN A, A^1, B *S. erythrochromogenes*	Syrupy oil. Acidic. Negative biuret, xanthoproteic, Sakaguchi, Molisch, Fehling, Tollens, Seliwanoff, Foulger, picric acid, doubtful nindydrin; positive nitroprusside, iodine azide. $C_7H_8O_3$.	230 (water) for pure sample	Also active against tumour and some bacteria. *H.T.* 2.

1	2	3	4	5
*59	MYCOMYCIN *Nocardia acidophilus*	Colourless needles. Highly unsaturated carboxylic acid; acetylenic. $C_{13}H_{10}O_2$. 75 dec. explosively	267, 281 (ether) —130(c, 0.4 EtOH, 25°)	Also active against gram+ bacteria. *H.T.* 2
60	PA 132 *Streptomyces* sp.	Colourless amorphous powder, darkens on standing. Unstable as free lactonic acid. Positive $KMnO_4$, Br_2-CCl_4; negative $FeCl_3$ (yellow green), Fehling, 2, 4-dinitrophenylhydrazine, Tollens, alc. $AgNO_3$, NA hypoiodite. $C_{16}H_{18\text{-}20}O_5$.	218.5 (MeOH) -161 (c, 1% MeOH, 25°)	Also active against gram +, gram- bacteria protozoa. *Antibiot. Ann.* **1956-57**, 672, 676.
61	MYCOTICIN *S. ruber.*	Yellow cryst. Fluoresces in U. V. Negative Br, $KMnO_4$; positive Brady, reduces ammonical $AgNO_3$. $C_{18}H_{30}O_5$.	210 236, 363	*H.T.* 2
62	CHRYSOMYCIN *Streptomyces* sp.	Slender greenish yellow needles or rods. Neutral. Photo-sensitive when dry. $C_{22}H_{20}O_7$. 255-260 dec. (micro-block)	247, 287, 390-400. +16 (c, 1 acetic acid, 22°)	Gram+ bacteria also inhibited. Active against phage. *H. T.* 2
63	OLIGOMYCIN B *Streptomyces* sp. resembling *S. diastatochromogenes*	Platelets. Oligomycins A, B, C closely related in structure. $C_{22}H_{36}O_6$. 160-61; on standing for several months m.p. changes to 169-70. The higher m.p. material on recrystallization from MeOH gives lower m.p. compound.	225 (EtOH) and 295 at higher conc. —49.5 (c, 1.03, MeOH, 23·5°);—46·4 (c, 0·76, dioxane 23·5°); 48·4 to —48·3	Fungi only. *J. Am. Chem. Soc.* **80**, 6092 (1958) *H.T.* 2
64	OLIGOMYCIN A *Streptomyces* sp. resembling *S. diastatochromogenes*	Long needles (I), hexagonal cryst. (II). Oligomycins A, B, C are closely related in structure. $C_{24}H_{40}O_6$. I: 140-41, II: 150-51 dec.	225 (EtOH) and 285 at higher conc. II: -54·5 (c, 4·4, dioxane, 22°)	Fungi only. *J. Am. Chem. Soc.* **80**, 6092, (1958). *Antibiot. and Chemother.* **9**, 287 (1959). *H. T.* 2.
65	OLIGOMYCINS *Streptomyces* sp. resembling *S. diastatochromogenes*	Colourless, hexagonal cryst. Negative phenolic, $FeCl_3$, digitonin. Positive 2,4-dinitrophenylhydrazine; slowly decolourizes Br in CCl_4 and $KMnO_4$ in acetone. $C_{25}H$ 40-42 135·8-136·3 uncor.	225, 232 (MeOH) —51 (c, 3 MeOH, 25·8°)	Fungi only *H. T.* 2.

1	2	3	4	5
66	RAMNACIN *Streptomyces* sp.	Does not decolourize Br in CCl_4; positive test for benzenoid structure. Very stable. $C_{26}H_{43}O_8$. 235		Also active against gram+ bacteria. *H.T.* 2. *Ann. Biochem.* **17**, 91 (1957)
67	OLIGOMYCIN C *Streptomyces* sp. resembling *S. diastatochromogenes*	Rods. Oligomycins A, B, C closely related in structure. $C_{28}H_{46}O_6$. 198-200	225, 232 ± 0·5 with inflec. at 220, 240 ± 0·5, 285 (A and C), 295 (B).	Fungi only *J. Am. Chem. Soc.* **80**, 6092, (1958). *H.T.* 2.
68	CABICIDINE *S. gougerotii*	Yellow columnar cryst. $C_{35}H_{60}O_{13}$. 225	-135 (at 34°)	*A. niger* inhibited. *C.A.* **53**, 22365f (1959)
69	NIGERICIN *S. violacioniger*	Colourless needles (Na salt). Monobasic acid. $C_{39}H_{69}O_{11}$ Na salt: 246-54 uncor.		Gram+ bacteria also inhibited. *H.T.* 2
70	IMOTICIDIN *S. albus*	Colourless, long prisms. Negative Sakaguchi, ninhydrin, xanthoproteic, biuret, Millon, Tollens. C 64·71, H 9.50, H_2O 7·63, ash 3.16. 245-46	No characteristic absorption.	*Corticium centrifugum* inhibited *C. A.* **53**, 20268ei (1959)
71	FLAVOFUNGIN *S. flavofungini*	Yellowish green, fine needles (*See* 18a)	263, 368	*Nature* **181**, 908 (1958). *Arnz. Forsch.* **9**, 178 (1959)
72	ROTAVENTIN *S. reticuli*	White thin leaves turning yellow at 130. No ppt. with flavianic, phosphotungistic, picric acids, methyl orange, Pb acetate, Ca, Hg, Ba chlorides. Red colour conc. H_2SO_4 170-75 dec.		*H. T.* 2
		Compounds Containing C, H, N, O		
73	ENTEROMYCIN (Seligocidin?) *S. albireticuli*	Faint yellow cryst. Contains COOH, $COCH_3$. $C_6H_8N_2O_5$	300-320	Also active against gram+ bacteria. *C. A.* **51**, 10643i (1957). *C. A.* **52**, 9529g (1958)
74	SELIGOCIDIN (Enteromycin?) *Streptomyces* sp. resembling *S. roseochromogenus*	Positive Sakaguchi, ninhydrin, negative biuret.	304	Also inhibits *Actinomycetes* and some gram + bacteria. *H. T.* 2

1	2	3	4	5
75	ABIKOVIROMYCIN (Latumcidin) *S. rubescens*, *S. reticuli* var. *latumcidicus*	White needles. Positive diazo, Baeyer, Br tests; negative $FeCl_3$, Fehling, Tollens, Pilotys, Ehrlich, Sakaguchi, ninhydrin, Molisch, biuret, xanthoproteic. $C_{11}H_{13}NO_2$ 120-25 dec.	230-240, 334-340 (0.1N HCl); 244-246, 280-291 (0.1 NaOH); 231-236, 334-338 (0.1N H_2SO_4)	Also active against bacteria *J. Antibiot.* (*Jap.*). **11A**, 6, 231 (1958.).
76*	1, 6-DIHYDROXY PHENAZINE *S. thioluteus*	Golden yellow prisms. Pigment pH indicator. $C_{12}H_8N_2O_2$ 274	272, 372, 440-445 (MeOH); 291, 520-530 (0.1N NaOH)	*J. Antibiot.* (*Jap.*) **12A**, 17 (1959).
77	CAERULOMYCIN *S. caeruleus*	Colourless cryst. Amphoteric. Red colour with $FeCl_3$, deep colour with ferrous salts in mineral acid soln. typical of α, α'- dipyridyl. $C_{12}H_{11}N_2O_2$ 175.	240, 285.	Also weakly active against some bacteria *Canad. J. Microbiol.* **5**, 317 (1959)
78	TOYOKAMYCIN *S. toyokaensis*	Colourless needles. Weakly basic. Negative Fehling, biuret, $FeCl_3$, positive ninhydrin. $C_{12}H_{14}N_5O_4$. 239-43 for monohydrate.	230, 279, 339. 230, 277 (water); 255, 273 (0.1N HCl); 233, 280 (0.1N NaOH)	Yeast like organisms, *Myco. tuberculosis* inhibited. *H. T.* 2. *J. Antibiot.* (*Jap.*) **9A**, 60 (1956). *C. A.* **52**, 15846g (1958)
79	HYGROSCOPIN A *S. hygroscopicus*	Oil. B. P. $_{0.003}$ 64.	235	Also active against *Myco. tuberculosis* and influenza virus *H. T.* 2
80*	ELAIOMYCIN *S. hepaticus*, *S. galaticus*	Light yellow, neutral oil. Negative $FeCl_3$, periodic acid xanthate, hydroxamate, Benedict, Na nitroprusside, ninhydrin, Sakaguchi, Ehrlich. $C_{13}H_{26}N_2O_3$	237.5 + 38.4 (c, 2.8 absol. EtOH, 26°)	*H. T.* 2. *J. Am. Chem. Soc.* **80**, 6088 (1958); **81**, 1435 (1959).
81	ANISOMYCIN *S. griseolus*, *S. roseochromogenus*	Long white needles. Basic. $C_{14}H_{19}NO_4$ 140-41.	224, 277, 283 (EtOH)-30 (c, 1 MeOH, 25°)	Some fungi, bacteria and protozoa inhibited. *H. T.* 2
82	BLASTCIDIN S *S. griseochromogenes*	White needle cryst. Negative $FeCl_3$, Fehling, Tollens, Na nitroprusside, triphenyl-tetrazolium chloride, bromonitroso, maltol, Millon, Ehrlich, Sakaguchi, Molisch, biuret, xanthoproteic, Graf's aldehyde, ammoniacal $AgNO_3$, ninhydrin. $C_{14}H_{20}N_6O_5$ 235-36 dec.	275 (0.1N HCl); 266-270 (0.1N NaOH) +108.4 (c, 1% water, 11°)	Inhibits *Pirucularia oryzae* specifically, and some bacteria. *J. Antibiot.* (*Jap.*) **11A**, 1, (1958)
83	FERMICIDIN (Fermizin) *S. griseolus*, *S. griseus*	Colourless needles. $C_{11}H_{21}NO_4$. 93-94, 96-98	290 +52.3 ± 1.5 (c, 0.65 water, 18°)	Also inhibits protozoa and viruses. *H. T.* 2. *C. A.* **54**, 834a, (1960)

1	2	3	4	5
84*	ACTIPHENOL *Streptomyces* sp.	Colourless needles. Structure related to Cycloheximide, Streptovitacins. $C_{15}H_{17}NO_4$ 199-200	215, 264, 354 (alc.); 229, 300, 375 (0.1 N NaOH) O (c, 1.89 tetrahydrofuran)	*Helv. Chim. Acta.* **42**, 1523 (1959).
85	MONILIN *S. sakaiensis*	Colourless needles. Positive ninhydrin, Sakaguchi. Thermostable, $C_{15}H_{20}N_6O_3$ 235-38 dec.	230, 280 (aq. soln.)	*C. A.* **52**, 20918i (1958).
86*	CYCLOHEXIMIDE (Actidione) *S. griseus*, *S. noursei*	Colourless plates. Weakly acidic. $C_{15}H_{23}NO_4$ 109-11, 112, 115-16.5, 120	—2.8 (c, 9.6 MeOH, 25°) -6.8 (c, 2 water, 25°)	*H. T.* 2, *J. Am. Chem. Soc.* **80**, 1261 (1958). *Chem. Pharm. Bull.* **6**, 328 (1958). **7**, 259 (1959). *Bull. Soc. Chim.* (1955), 1316
87*	NARAMYCIN B *Streptomyces* sp.	Colourless plates. Stereoisomer of Cycloheximide (86). $C_{15}H_{23}NO_4$ 109-110	292.5, sh. 232 +48.8 (c, 1 water, 9°)	*Chem. Pharm. Bull.* **6**, 328 (1958), **7**, 259 (1959)
88*	STREPTOVITACINS *S. griseus*	A: Orthorhombic (I), monoclinic. (II). Nonhygroscopic. (I) transformed to (II) at 147°. Cycloheximide type structure. 3-[2 (4-hydroxy-3, 5-dimethyl - 2 - oxycyclohexyl) - 2-hydroxyethyl]-glutarimide. B is isomer. $C_{15}H_{23}NO_5$. A (II): 156-61. B: 124-28.	A, B end absorption only. Optically active	Limited antifungal activity. Antitumour. *Antibiot. Ann.* **1958-59**, 547,555, 560, 565. *J. Am. Chem. Soc.* **81**, 2595, (1959)
89	HYGROSCOPIN B *S. hygroscopicus*	Oil. $C_{15}H_{28}N_2O_3$ B. $P_{0.008}$ 70	233 (EtOH). —38.8 (MeOH, 14°)	Also active against Yoshida sarcoma and influenza virus *H. T.* 2
90	HYGROMYCIN B *S. hygroscopicus*	Polyhydroxybase. Positive Molisch, anthrone. Negative Benedict, Fehling. $C_{15}H_{28}N_2O_{9\text{-}10}$ About 180	No U. V. absorption.	Also active against gram+gram,— antiparasitic effect in swine. *J. Am. Chem. Soc.* **80**, 2714 (1958)
91*	STREPTIMIDONE *Streptomyces* sp.	Colourless needles (from acetone-isopropyl ether). Becomes yellow, oily after two weeks in sunlight at room temp. 3-(2-hydroxy-7-methyl-5-methylene-4-oxo-6-nonenyl-glutarimide. $C_{16}H_{23}NO_4$ 272-73.	232, 291, (MeOH); 231, 289 (water) +238 (c, 0.5% water, 28°); +245 (c, 0.5% $CHCl_3$, 27°)	Similar to Cycloheximide in chemical, biological properties. *J. Am. Chem. Soc.* **81**, 5500 (1959)

1	2	3	4	5
92	STREPTOTHRICIN *S. lavendulae*, *Streptomyces* spp.	HCl and sulphate white powder. Basic. Reduces Tollens, neutral $KMnO_4$, boiling Fehling. Positive biuret, ninhydrin, Pauly. Negative $FeCl_3$, nitroprusside, Molisch, Sakaguchi, Schiff, Hopkins-Cole, Millon. $C_{20}H_{36}N_8O_9$. Reineckate: 192-94 dec., helianthate: 220-30 dec.	End absorption only. HCl: —51.3 (c, 1.4 water, 25°)	Also active against gram+, gram— bacteria. *H. T.* 2.
93	ANTIBIOTIC *Streptomyces* sp.	Streptothricin group. Negative ninhydrin, Pauly, Sakaguchi, Hopkins-Cole, Fehling, Tollens, Molisch, hydrophilic phthalaldehydes. Acid hydrolysis gives amino acid, sugar.		Also active against gram+, gram— bacteria. *Acta Chem. Scand.* **11**, 755 (1957)
94	ANTIBIOTIC 136 *S. lavendulae*	Basic. Streptothricin-like.		Also active against gram+, gram— bacteria. *Arch. Biochem.* **15**, 215 (1947)
95	PLEOCIDIN *Streptomyces* sp. resembling *S. lavendulae*	White hygroscopic powder. Basic. Related to Streptothricin (92). Maximum stability at pH 4-6.		Also active against gram+, gram— bacteria. *H. T.* 2
96	MYCOLUTEIN *Streptomyces* sp. (Mycelium)	Bright yellow tablets (from MeOH and benzene). Dissolves in conc. H_2SO_4 giving olive colour turning dark red. Decolourizes $KMnO_4$ in acetone in cold and Br. soln. in MeOH with evolution of HBr. Positive for aromatic nucleus. $C_{22}H_{24}NO_6$. 157-58.	254, 345	*H. T. 2.*
97	CEREVIOCCIDIN *Streptomyces* sp. resembling *S. cacaoi*	Colourless needles. Positive Janovsky; negative Tollens, biuret, Fehling, ninhydrin, Sakaguchi, maltol, glucosamine. $C_{22}H_{39}N_5O_4$. 249-50 dec. Sublimes 217-18.	No absorption.	*H. T. 2* Yeasts only.
98	VENGICIDE *S. vendargensis*	White. Produced along with Oxytetracycline. $C_{24}H_{29}N_{10}O_9$ 241.5-243	233.5, 273.5 (0.05N HCl) —51.6 (0.1N HCl, 20°)	*C. A.* **51**, 10009a (1957)
99	EULICIN *Streptomyces* sp. resembling *S. parvus.*	HCl: colourless. Basic. Positive Sakaguchi. $C_{24}H_{52}N_8O_2$ Helianthate: 139		*H. T.* 2. *J. Am. Chem. Soc.* **80**, 5173 (1958)

1	2	3	4	5
100	AYFACTIN *S. aureofaciens* (Mycelium)	$C_{25}H_{35\text{-}36}NO_7$		*C. A.* **53**, 1646d (1959)
101	BLASTMYCIN *S. blastmyceticus*	White needles. Resembles Antimycin A (106). Positive $FeCl_3$, diazo, biuret. Negative ninhydrin, Molisch, Fehling, Tollens, Ehrlich, Millon, conc. H_2SO_4. $C_{26}H_{36}N_2O_9$. 167 uncor., 168-69.	225, 321 (NaOH); 222, 245 (with excess equivalent of base) +77.4 (c, 1 MeOH, 15°)	*J. Antibiot.* (*Jap.*) 10A, 39 (1957); **11A**, 122, 254 (1958)
102	ANTIMYCIN A_3 *Streptomyces* sp. *S. kitazawaensis*	$C_{26}H_{36}N_2O_9$. Differs from Blastmycin (101) in I. R. spectra. 167-68.	228, 325 (MeOH) + 84 ($CHCl_3$)	*J. Antibiot.* (*Jap.*) **11A**, 32 (1958)
103	ANTIBIOTIC *Streptomyces* sp. resembling *S. albus*	Isomaltose-like. $C_{26}H_{36}N_4O_9$ 79-82 (for hydrate)	+140.9 (c, 1 water, 20°)	Also active against gram+, gram— bacteria. *J. Antibiot.* (*Jap.*) **7B**, 51 (1951)
104	ANTIMYCIN A—Mixture *Streptomyces* sp. *S. kitazawaensis*	White needles. $C_{27}H_{38}N_2O_9$. 138-39. (Cf. 106)	228, 325 (MeOH) + 66.8 ($CHCl_3$)	*J. Antibiot.* (*Jap.*) **11A**, 32 (1958)
105	VIRTOSIN *S. olivochromogenes*	Colourless needles. Positive Fehling, Sakaguchi Negative ninhydrin, maltol $C_{27}H_{40}N_2O_9$ 142.5-143	+80 ±0.5 (c, 1% acetone, 18°)	*C. A.* **52**, 20919b (1958)
106	ANTIMYCIN (Antimycin A) *Streptomyces* sp. *S. kitazawaensis*	Colourless cryst. Weakly acidic. Positive $FeCl_3$, Gibbs phenol, Folin, diazobenzene-sulfonic acid, xanthoproteic. Negative Molisch, ninhydrin, anthrone, Benedict, Hopkins-Cole, Millon, biuret, Ehrlich, fuchsinaaldehyde, 2, 4-dinitrophenylhydrazine, conc. H_2SO_4. $C_{28}H_{40}N_2O_9$ 139-40, 148-149.5, 149. 8-150·2, 141-42, 140.5-141.5.	230, 320, 245, 347, 225, 320 (EtOH) +64.8 (c, 10 $CHCl_3$, 25°), + 77.2 + 1.0 (c, 3.57 $CHCl_3$, 25°)	*H. T.* 2. *Proc. Chem. Soc.* (1950) 22. Strong, F. M. Topics in microbial chemistry. Wiley, 1958, p. 1.
107	ANTIMYCIN A_1 *Streptomyces* sp. *S. kitazawaensis*	$C_{28}H_{40}N_2O_9$ 147-48.	228, 330 + 74 ($CHCl_3$)	*J. Antibiot.* (*Jap.*) **11A**, 32 (1958)
108	RACTINOMYCIN A *S. phaeochromogenus*	Yellow or orange needles. Negative ninhydrin, biuret, Millon, Positive $FeCl_3$, Tollens; reddish violet turning blue with conc. H_2SO_4; orange turning violet on heating with HCl; violet turning reddish violet with NaOH. Aq. soln. yellow at acid and violet at alk. pH. $C_{33}H_{30}N_3O_{14}$. Turns brown at 157-58, blackens 205.	245, 440-450 (MeOH)	Inhibits some fungi, gram+, gram— bacteria, Ehrlich carcinoma, HeLa cells. *H. T.* 2. *J. Antibiot.* (*Jap.*) **10A**, 115 (1957)

1	2	3	4	5
109	MUSARIN *Streptomyces* sp.	Light yellow powder. Acid. Negative Molisch, Millon, biuret, H_2SO_4 in acetic acid, or with I, Salkowski, Liebermann, murexide. Positive Axenfeld; ppt. with mineral acids, $BaCl_2$, $HgCl_2$, Cu acetate. $(C_{35}H_{60}N_2O_{14})n$.	240, 267 (EtOH) +35.1 ± 1.6 (c, 1.21 MeOH, 20°), + 38.7 ± 2.7 (c, 0.736 MeOH, 20°)	Also active against gram+ bacteria. *H. T.* 2
*110	ANTIBIOTIC 1-81d-1s *S. albus*	Dark red in cold conc. H_2SO_4, lavender with 85% H_3PO_4. Reduces $KMnO_4$ slowly. C 63.1, H 8.87, N 1.92, O 26.48. $C_{38}H_{63\text{-}65}NO_{12}$. 140-41.	244, 284 —29.8 (1.14% Me_2CO, 22°)	Also active against gram+ bacteria, influenza virus PR8 in chick embryo. *C. A.* **52**, 1557h, 1957.
111	AMIDOMYCIN *Streptomyces* sp.	Colourless needles. Neutral. Polypeptide, contains 4 moles each of D(—) valine, D (—) *a*-hydroxyisovaleric acid linked alternately by ester and amide bonds to form 24-membered ring. Related to Valinomycin. $C_{40}H_{68}N_4O_{12}$. 192.	+19.2 (c, 1.2 EtOH, 25°	*Canad. J. Microbiol.* **3**, 953 (1957). *Canad. J. Chem.* **35**, 1109 (1957.) *Bact. Proc.* (1957) 70
112	ACTINOMYCIN A *Streptomyces* spp. *S. antibioticus*	Red-vermillion platelets. Weakly basic chromopeptide, quinonoid. Contains L-threonine, sarcosine, L-proline, D-valine, N-methyl-L-valine. $C_{41}H_{58}N_8O_{11}$ 250, 252 dec.	230-250, 450 (EtOH) —320 ± 5 (EtOH, 25°)	Inhibits some fungi and gram+ bacteria. *H. T.* 2
113	CINERUBINE A *S. antibioticus* *Streptomyces* spp.	Dark red microcryst. powder. 1, 4, 5-Trihydroxyanthraquinone. $C_{43\text{-}45}H_{57\text{-}61}NO_{18}$. 155-58; solidifies at 160-188 in rosette needles which disappear above 249.	235, 259, 294, 473, 487, 497, 518, 533 (96% EtOH); 508, 545, 588 (pyroboroacetate in acetic anhydride on warming)	Inhibits some fungi, gram+ bacteria influenza virus, tumour. *Chem. Ber.* **92**, 1867 (1959).
114	CINERUBINE B *S. antibioticus* *Streptomyces* sp.	Orange red platelets. 1, 4, 5-Trihydroxyanthraquinone. $C_{43\text{-}45}H_{57\text{-}61}NO_{18}$ 168-78 solidifies on further heating and disappears at 240-43; 180 (capillary)	235, 258, 294, 473, 488, 497, 519, 532 (96% EtOH); 550, 595 (pyroboroacetate in acetic anhydride in cold); 508, 545, 589 (pyroboroacetate in acetic anhydride on warming)	Activity as for Cinerubin A (113) but less active. *Chem. Ber.* **92**, 1867 (1959)
115	MELANOSPORIN *S. melanosporus* sive *melanosporofaciens*	Yellowish white amorphous solid. Negative $FeCl_3$, ninhydrin; acid hydrolysate gives 3 ninhydrin positive spots. Yellow with conc. H_2SO_4. $C_{56\text{-}63}H_{105\text{-}117}N_3O_{30\text{-}22}$. 132-34.	230 +30 (c, 1.578 MeOH, 20°)	Also active against gram+ bacteria. *G. Microbiol.* **7**, 207 (1957)

1	2	3	4	6
116	DATEMYCIN *Streptomyces* sp.	Colourless powder. Positive ninhydrin on hydrolysis. Negative Hopkins-Cole, xanthoproteic, Sakaguchi, Millon, Elson Morgan, Molisch, Fehling, Ag. mirror. Pseudo-positive biuret. $C_{58}H_{102}N_4O_6$ 197 dec.	247 −43·7 (water, 15°)	Inhibits *C. albicans*, *C. paracrusei* only *CA.* **54**, 832f (1960)
117	PHYTOSTREPTIN *Streptomyces* sp. (variant of strain producing Phytoactin (118))	Polypeptide containing same amino acids as Phytoactin (118). Water sol., dialyses through cellophane into water.		*Phytopath.* **47**, 539 (1957)
118	PHYTOACTIN *Streptomyces* sp.	Polypeptide similar to Phytostreptin (117); contains valine, *a*-alanine, proline, leucine (or isoleucine), arginine, glycine, serine. Free carboxyl group. Slightly sol. water, dialyses through cellophane into aq. MeOH.		*Phytopath.* **47**, 539 (1957)
119	ALBOFUNGIN *S. albus* var. *fungus*	Contains C, H, N, O. 190	240, 255, 305, 375	Also active against gram positive bacteria. *Antibiotiki* **4**, No. 6, 5, 11 (1959)
120	FLAVENSOMYCIN *S. tanaschiensis*	Pale yellow, odourless, tabular cryst. Positive Ehrlich, for carbohydrates; negative for proteins. 152] 2.	251 (20 mmg/ml in MeOH)	Also active against *Musca domestica*, *Locusta migratoria* *Nature* **179**, 1307 (1957)
121	ANTIBIOTIC 1268 *Streptomyces* sp.	Stable in aq. soln. at neutral pH, and at room temp. less than 12° Stable to light.	251	Also antimitotic effect on *Allium cepa* root meristem *C.A.* **51**, 18158a (1957)
122	SUBSTANCE A *Streptomyces* sp. resembling *S. fungicidicus*	Prisms. Negative ninhydrin, Sakaguchi, biuret, Millon, Molisch, Seliwanoff, Fehling, $FeCl_3$. Orange in 40% H_2SO_4. C 61·47, H 7·41, N 51 .5, 0 25·93 164-70	212, 260	Inhibits fungi only. *C.A.* **51**, 18101b (1957)
123	ANTIBIOTIC *Streptomyces* sp.	Colourless needles. C 49·33, 49.47, H 4·9, 4.56, N 23·75, 24·14. 238-39	230·5, 278-279 -51 (c, 0·13 water, 35°)	Inhibits *Candida* spp. specifically. *Ann. Rept. Takeda Res. Lab.* **16**, 28 (1957)

1	2	3	4	5
124	ANTIBIOTIC *Streptomyces* sp.	Reduces Tollens, Fehling; weakly positive ninhydrin, biuret, no reaction with Schiff and Molisch. N content of hydrochloride 13·49%.	—32°[5] (aq. soln.)	*C.A.* **51**, 11429h (1957)
125	PA 86 *S. rimosus*	C 60·3, H 8·3, N 3·41, 027·99 230-50 dec.	Optically inactive	*C.A.* **49**, 7199i (1955)
		Compounds Containing C, H, N, O, S		
126	THIOAURIN (HA-9) *Streptomyces* sp. related to *S. lipmanii*	Yellow cryst. Negative $FeCl_3$. $C_7H_6N_2O_2S_2$ or $C_{14}H_{12}N_4O_4S_4$ 179-81, 175-80.	232, 370. O (in glacial acetic acid)	Inhibits gram+, gram—bacteria and weaker activity against fungi and yeast. *H.T.* 2
127*	HOLOMYCIN *S. griseus*	Orange yellow, radial leafy cryst. Neutral, lipophilic. de-N-methyl-thiolutin. $C_7H_6N_2O_2S_2$ 264-71 dec.	250, 305, 390 (broad)	Also active against gram+, gram—bacteria and protozoa. *Helv. Chim. Acta* **42**, 563 (1959)
128*	THIOLUTIN *S. albus*	Brilliant yellow needles. Neutral. $C_8H_8N_2O_2S_2$. 270 dec.	245, 315, 365; 250, 311, 388 Optically inactive	Also active against gram+, gram—bacteria *H.T.* 2
129*	AUREOTHRICIN *S. celluloflavus*	Golden yellow needles. Similar to Thiolutin (128) $C_9H_{10}N_2O_2S_2$ 256-57 dec.	248, 312, 388. Optically inactive	Also active against gram+, gram—bacteria *H.T.* 2
130	ANTIBIOTIC 4738-A *S. cyanoflavus*	Yellow needle like cryst. Similar to or identical with Aureothricin (129) 255-56 dec. (in sealed tube)	245, 310, 390	*J. Antibiot.* (Jap). **11A**, 145 (1958)
131	ALTHIOMYCIN *S. althioticus*	White needles. Positive ninhydrin, Tollens. Negative $FeCl_3$, Fehling, Sakaguchi. $C_{15}H_{14}N_4O_6S_2$. 172-74 dec., browns 120-60.	220-223, 285-290 (methyl cellosolve), 235, 300-305 (0·03N NaOH-1% methyl cellosolve) +20·3 (c, 1·33% in methyl cellosolve, 20°)	Moderate activity against fungi. Also active against gram+, gram-bacteria *J. Antibiot.* (*Jap.*) **10A**, 195 (1957)
131a	SULFOCIDIN *Streptomyces* sp.	White amorphous powder. Neutral. Dissolves in conc. H_2SO_4 with dark brown colour. Negative nitroprusside, azide-iodine, ninhydrin, Sakaguchi, maltol, $FeCl_3$, Fehling, biuret, nitrochromic acid, 2, 4-dinitrophenylhydrazine, anhydrous aluminium chloride. Decolourizes $KMnO_4$ in acetone in cold. C 62·0, H 7·5, N 3·1, S 3·6.	End absorption with sh. at 285 —58·5 (c, 0·51 $CHCl_3$, 25°)	Also active against gram+, and gram— bacteria. *Antibiot. Ann.* 1957-58, 886

1	2	3	4	5
132	UNAMYCIN B *S. fungicidicus*	Colourless needies. Negative $FeCl_3$, Sakaguchi, Fehling, Tollens, Ag mirror, Schiff; green with Molisch. Prussiate soln. changes yellowish with Millons 236-38.	236, 273 (0·05N HCl) -43 (1% MeOH, 15°)	*C.A.* **54**, 831c (1960)
		Other Antifungal Antibiotics		
133	BLASTCIDINS A, B, C. *S. griseochromogenes*	A: Light yellow powder. B: Colourless liquid, B.P. 0·001 36°. C: Red brown powder.	A: 216.	Inhibit *Piricularia oryzae* specifically. *Bull. Agric. Chem. Soc. Jap.* **19**, 181 (1955)
134	NIGER FACTOR *Streptomyces* sp.		258	Inhibits *Aspergillus niger*. *C.A.* **52**, 13159h (1958)
135	S 39 *Streptomyces* sp. resembling *S. albus*	White cryst. Water sol. 97-99.	260	Anti-*Candida*. *Hindustan Antibiot. Bull.* **2**, 7 (1959)
136	ANTIBIOTIC E 150 *Streptomyces* sp.		About 260	*J. Antibiot. (Jap.)* **8A**, 189 (1955)
137	ANTIBIOTIC *S. mashuensis* (Mycelium)	Powder. Stable at 100° for 1 min. at pH 8·5 or 2.	335	
138	ENDOMYCIN *Streptomyces* sp, related to *S. albus. S. endus.*	Acidic.	226, 232.	Also active against gram+ bacteria, protozoa. *H.T.* 2.
139	ANTIBIOTIC *S. phaeochromogenus*	White cryst. 151-53.	225, 343.	Weakly antifungal. *J. Antibiot. (Jap.)* **8A**, 132 (1955)
140	TRICHONIN *S. rubrieticuli*		225, 320-325 (MeOH)	*J. Antibiot. (Jap.)* **8A**, 189 (1955)
141	ALOMYCIN *Streptomyces* sp.	Tea coloured oil. Stable at 10° for 48 hrs.	270, 350 (small max.)	*C.A.* **52**, 8277i, 15642h, 15643a (1958)
142	ANTIBIOTIC *S. globisporus flaveclus*	Crude compound sol. lower alcohols, less sol. in diethylether, almost insol. in water.		Inhibits *C. albicans*, *Fusaria*. *Rev. Appl. Mycol.* **38**, 186 (1959)
143	ANTIBIOTIC *S. griseocarneus* (Mycelium)	No ppt. in MeOH, or EtOH with phosphotugstic, picric, flavianic acids, Pb acetate, $CaCl_2$, $HgCl_2$. Red colour with conc. H_2SO_4.		Inhibits fungi only. U.S. Patent 2, 617, 755, (1952)

1	2	3	4	5
144	D-SUBSTANCE *S. flavus*	White needles. Acidic. Sol. in organic solvents only. 124-25.		*Saccharomyces cerevisiae* inhibited. *J. Antibiot.* (*Jap.*) **6A**, 117 (1953)
145	EUMYCETIN *Streptomyces purpeochromogenus*	Colourless white needles. Positive $FeCl_3$, diazo. Negative biuret, Millon, ninhydrin, Molisch, Liebermann-Burchard, Sakaguchi, Rosenheim, Fehling; no colour with conc. H_2SO_4, HCl. 148-50.	302 (MeOH)	*H.T.* 2.
146	ANTIBIOTIC 720B *Streptomyces* sp. related to *S. olivochromogenus*	White needles. 169.5-170	No. U.V. max.	Inhibits *P. chrysogenum*
147	ANTIBIOTIC G. S. I *Streptomyces* sp.	Inactivated by U.V., and oxidation. Stable to autoclaving.		Inhibits plant pathogenic fungi. *Phytopath.* **49**, 112 (1959)
148	ANTIBIOTIC *Chainia antibiotica*			Moderate activity against gram+ also. *Nature* **176**, 934 (1956)
149	HUMIDIN *S. humidus*			Inhibits *Hypochnus sasakii, Colletorichum lagenarium, Ustilago zeae.* *Ann. Rept. Takeda Res. Lab.* **17**, 31 (1958)
150	HELIXIN *Streptomyces sp.*	Components A, B, C, D; B identical with a component of Endomycin (138). Negative Molisch. ninhydrin, Hopkins-Cole, xanthoproteic, Millon, $FeCl_3$. Deep red in EtOH soln. Amorphous ppt. in aq. soln. below pH 4-5.		Also active against some gram+ bacteria. *H.T.* 2.
151	MICROCINS A, B. *Micromonospora sp.*	A: Reddish-purple powder. Neutral. Insol. water. B: Yellowish red powder. Acidic. Slightly sol. water. Both give negative Molisch.		Moderate activity against fungi. Also inhibit gram+bacteria. *H.T.* 2.
152	MOLDIN *S. phaeochromogenus*	Positive Molisch, $FeCl_3$; negative biuret, ninhydrin, Tollens, Sakaguchi.	370	*H.T.* 2.

CHART 1: INDEX TO MELTING/DECOMPOSITION POINTS (Range in °C)

M.P.	70—75	75—80	80—85	85—90	90—95	95—100	100—105	105—110	110—115	115—120
S. No.	59*	103			83	83 135	40	86 87	42 86	86
M.P.	120—125	125—130	130—135	135—140	140—145	145—150	150—155	155—160	160—165	165—170
S. No.	75 144	88		65 106 99 104	64 106 81 110 105	106 107 145	64 120 139	45 113 88 96	4* 60	101 102 122 146
M.P.	170—175	175—180	180—185	185—190	190—195	195- 200	200—205	205—210	210—215	215—220
S. No.	52* 131 72 114	77 114 126	29* 90		92* 111 119	26 116 67 84	1*	27		36 37 38
M.P.	220—225	225—230	230—235	235—240	240—245	245—250	250—255	255—260	260—265	265—270
S. No.	92*	68	10	20 85 32 123 66 125* 82 132	32 78 98	69* 70	112	62 129 130	35	127
M.P.	270—275	275—280	280—285	285—290	290—295	295—300	300—305	305—310	310—315	315—320
S. No.	76 91 128									

Remarks on aesterick-marked numbers: (1) Decomposes approx. at 200 with definite m.p. (4) Gradual dec. above 160 without melting by 250. (29) Melts slowly 180-230 with frothing. (52) Gradual dec. over 170. (69) Na salt. (92) Reineckate, m.p. 192-94; helianthate, m.p. 220-30 dec. (99) Helianthate. (125) 230-250 dec.

CHART 2: INDEX TO U. V. ABSORPTION MAXIMA*

λ Max (mμ)	0	1	2	3	4	5	6	7	8	9
210	61		122			85	133		10 16	
220		131	1 101		81	63 101 64 106 65 139 67 140	11 138		102 104 107	84
230	4 106 58 115 78 123 85	91	11 126 65 138 67 91	75 78 89 98	43 53 55	75 114 79 131 113	61 132	80		
240	77 119 109 128 112		50	33 36	110	75 108 101 128 106 130	116	62	129	
250	127 128	120			96	78 119			114 134	113
260	122 136 135			18a	84			59 109	82	
270	141		76	78 132 98		82		15 81 78	123	1 78 8
280	4 85 78	16 59	43	81	110	64 77	45	62 131	14 55 53	16 91
290	1 15 5 18 6 42 7 75 11 83 13	2 12 4 78 8 91 9	4 10 16 87		33 36 113 114	63				
300	84	14	131 145	1 15 6 16 12 19	2 11 3 13 4 74 8 127	4 16 5 18 7 23 9 39 11 42 119	10	14		

*For a broad maximum, approximately the middle wavelength is marked in the chart, e.g. for λmax 334-340 for Abikoviromycin the S. No. 75 is posted in the chart at λ max 337.

CHART 2 (*Contd.*)

λ max (mμ)	0	1	2	3	4	5	6	7	8	9
310	73 130	27 128	129			128	7	6 16 19 23	1 20 4 22 5 25 8 28 15 18	2 12
320	4 16 5 24 9 42 10 106 13	21 39 101	26 31 32 140	27	29	30 104				
330	21 107		19	20 24 22 25 23 28		33 36 137	76	75	26 31 32	27 29 78
340	30 39 34 40 37 41 38 51	40 42		139	54	43 96	45	106	53 55	19
350	22 141 23 24	20 28 21 25	41		84	26 33 36	31 37 32 38 34 39	27 53 55	29 44 30 50 40 51 42	57
360	40 47 43 48 44 48a 46	49	44 56	52 54 61	45 52	128			18a	
370	126		76	33 36		85 119	53 55	39 50 51	34 38 37 48a	40 44
380	40 47 42 48 44 57 46	41 44 49	52 56	43 45 52 54					128 129	
390	127 130					62		51	53 55	
400	47 50		44	44 48 46 53	44 49 57	43 48a 45	52 56		52	

CHART 2 (*Contd.*)

λ max (mμ)	0	1	2	3	4	5	6	7	8	9
410										
420										
430										
440				76		108				
450	112									
460										
470				113 114						
480								113	114	
490								113 114		
500									113	
510									113	114
520						76				
530			114	113						
540						113				
550	114									
580									113	
590						114				

CHART 3: INDEX TO EMPIRICAL FORMULAE

		0	1	2	3	4	5	6	7	8	9
0	C							73	58 127 126	128	129
	H							126 127		58 76 73 128	
	N		1 75 88 114 2 81 91 3 83 96 4 84 100 19 86 110 52 87 113	51 90 73 101 76 104 77 105 80 106 89	108 115	34 103 111 126 131	97	82 85		92 99 112	
	O			59 126 75 127 76 128 77 129 99	58 80 85 89	34 86 126 78 87 81 91 83 97 84 116	60 61 73 78 82 88	63 131 64 67 96 116	62 65 100	66	18a 104 90 105 92 106 98 101 103
	S			126 129 127 131 128		126					
10	C		75	76 78 77	59 80	81 83 82 126	84 87 131 85 88 86 90	60 91		61	
	H	59 129	77	126	75	78 131			84	60	60 81
	N	98									
	O	26 90	69 112	110 111	27 68	1 3 20 109 2 19 108				51 114 113	4
20	C	92		62 96 63 97		64 99 98	65 100	66 103 101	104 105	67 106	
	H	60 82 85 62 83			86 88 87 91	96		80		89 90	98
	O	52 115	155	115						110	

CHART 3 (*Contd.*)

		0	1	2	3	4	5	6	7	8	9
30	C	18a 34		26	108	1	2 68 27 109	3	19		69
	H	61 108				34	100	63 100 103 92 101		104	97
40	C	111	20 112		113 114	113 114	113 114	4 52			
	H	64 105 65 106	65	65	66			67		18a	1
50	C					51	115	115	115	115 116	115
	H			99	2	26			3 114 113	112 114 113	113 114
60	C	115	115	115	115						
	H	27 109 68 113 114	19 114 113		110	110	110			111	69
70	C										
	H				52				4	20	20
80	C										
	H	20		51							
90	C										
	H										
100	C										
	H			116			115	115	115	115	115
110	C										
	H	115	115	115	115	115	115	115	115		

4. Merck and Co., Inc.*

RAHWAY, N.J.

MERCK and Co., Inc., the "venerable aristocrat of the American pharmaceutical industry" has been a leader in many lines including antibiotics, vitamins, steroids and industrial chemicals. The Merck tradition of distinguished pharmacists and pharmaceutical manufacturers can be traced back almost three centuries in 1668, when Friedrich Jacob Merck took over the pharmacy "At the sign of the Angel" at Darmstadt in Germany. The German house found in U.S. a good customer for its chemicals. Eight years later land was acquired in Rahway, N.J., and a plant set up for manufacturing operations. Soon a long line of chemicals such as chloral hydrate, iodides, bismuth salts, acetanilide, narcotics, salicylates, alkaloids, disinfectants, photographic and reagent chemicals were steadily flowing into world markets. In 1927 the Weightman-Rosengarten firm of Philadelphia was acquired to give Merck a lead in quinine business. In 1953 the company merged its assets with those of Sharp and Dohme, Inc. The latter began as an apothecary shop in Baltimore in 1845 and rose to become a leader in the manufacture of several biologicals. The merger brought vaccines, blood plasma products, sulpha drugs and pharmaceutical specialities to Merck's product lines, together with the $4 million new Sharp and Dohme Research Laboratories and its fine research team at West Point, Pa.

Research facilities at Rahway were extended in 1933 with the establishment of laboratories including the Merck Institute for Therapeutic Research. Merck's traditional policy of backing with financial and moral support all scientific investigations and providing an atmosphere in which creative minds flourish, has been the back bone of the Company's successes and fortunes despite some costly failures.

VITAMINS TO ANTIBIOTICS

In 1934 in response to a request from Dr. R. R. Williams of the Bell Telephone Laboratories, Merck plunged into the production of vitamin B_1 from tons of rice bran. Dr. Williams established the structure of the vitamin and its synthesis followed. By 1937, Merck the first patent licensee, was making quantities of this vitamin from simple organic compounds. Other vitamins such as vitamin B_2, B_6, K and niacin were soon on the company's production lines. While the firm was investing heavily in equipment to produce the vitamins from natural materials, researchers including those at Merck laboratories, were evolving cheaper synthetic methods, making the equipment all obsolete.

The year 1948 is a landmark in the history of vitamins. Early that year Merck scientists isolated from liver vitamin B_{12}, the antipernicious anaemia factor, in pure crystalline form. Later the same year followed the announcement of the production of the vitamin by *Streptomyces griseus*, the organism which produces Streptomycin and other antibiotics. By February 1949 vitamin B_{12} from fermentation was on the market.

So well known for its research and large scale production potentialities it is no wonder that in 1941 Merck was among the first American pharmaceutical firms which Sir Howard Florey and Dr. N. C. Heatley approached with their penicillin producing strain for the large scale manufacture of the life-saving drug. Penicillin was not, however, the first antibiotic which the Merck scientists had to deal with. In 1939, when Dr. Dubos and co-workers of Rockefeller Institute announced the isolation of Tyro-

* Courtesy: Merck, Sharp and Dohme International, New York-7, N. Y.

thricin, Merck was interested and working on it gained experience in the techniques of production, isolation and extraction of antibiotics. The Company had the heaviest investment with $ 750,000 in the penicillin development project in which a score of British and American firms co-operated with $ 3 million and federal funds amounting to $ 350,000. The situation was challenging, new and development of the drug emergent under war conditions. All phases of the problem were attacked simultaneously. Stationery flask cultures expanded into batteries of cylindrical steel vessels for submerged fermentation. Studies on the composition of the fermentation medium, selection of high-yielding strains, and development of better methods of isolation and concentration of the antibiotic resulted in products of higher potency. Development microbiologists, chemists and chemical engineers worked up the pilot plant production and tackled such problems as proper design of agitators, proper aeration, construction details to minimize contamination and setting up optimum production conditions. Difficulties arising in the extraction of the labile penicillin were solved and rigid methods of aseptic handling of the product worked out. More facile chemical assay methods based on the chemistry of penicillin were devised. The failures, bottlenecks and uncertainties made progress extremely slow, but substantial supplies of penicillin were made available to the armed forces by D-day.

Studies on the chemistry of the penicillin molecule were continued. Remembering how synthetic methods outdated the natural extraction processes for the B vitamins, the Company invested heavily in researches for the synthesis of penicillin. The molecule would not, however, yield ; but the chemical studies helped further development work ; for instance, it paved the way for increasing penicillin production by incorporating into the fermentation medium certain moieties of penicillin.

The great success of penicillin as a therapeutic compound stimulated systematic screening of microorganisms for antibiotic production. Dr. Waksman, working at Rutgers University Experimental Station, turned up with Streptothricin active against gram negative organisms; Merck volunteered to produce sufficient quantities of the antibiotic in pilot plant for chemical and pharmacological investigations. Two years later in 1944 Dr. Waksman discovered Streptomycin and found it more active against gram negative organisms and also less toxic than Streptothricin. Merck agreed to produce quantities of the antibiotic for further testing. When the Mayo Clinic reported Streptomycin active against certain types of tuberculosis in animals, Dr. Waksman regarded the discovery as too important for any one producer. To establish commercial priority Merck put up a Streptomycin plant at Elkton, Va., in 1945 even before the actual process was worked out, was first to put the antibiotic on the market in 1946 and is still a leader in the line. As in the case of penicillin the research men had to tackle all phases of the program simultaneously, including the special pharmacological problems of Streptomycin side reactions and toxicity. Merck's selfless action to willingly give up sole right to produce the antibiotic enabled several companies to manufacture Streptomycin and helped to fight tuberculosis. By 1950 the antibiotic was in over production. Further researches on Streptomycin at the Merck laboratories resulted in the synthesis of Dihydrostreptomycin.

Novobiocin is another antibiotic resulting from Merck researches (also independently discovered at Upjohns). Introduced in clinical use four years ago, it is of value in the treatment of infections incited by organisms that have become resistant to other antibiotics.

OTHER FIELDS OF INTEREST

Sulphonamides : Merck scientists had a considerable part in the development of

sulphathiazole, the compound with which the American pharmaceutical industry effectively entered the race for better sulpha drugs. *Sulfasuxidine* and *Sulfathanilidine* (1942) with local action, and the sulphapyridines *Sulfadiazine*, *Sulfamerazine* and *Sulfamethazine* with systematic action against dysentery and ulcerations, are other Merck contributions.

Steroids : Merck's interest in steroids dates back to the 1930's when Dr. Edward Kendall of Mayo synthesized Compound A (one of the four compounds he had isolated from beef adrenal glands) with intermediates supplied by the Company. Although Compound A was biologically disappointing, Dr. Lewis Sarett at Merck's went on to synthesize Coumpund E after a many-staged and extremely expensive method. Cortisone thus introduced proved a landmark in the treatment of rheumatoid arthritis. Two years later partial synthesis of Compound F or hydrocortisone, which proved better than cortisone, was announced by Merck's chemists. Dexamethasone (*Decadron*) is another Merck contribution, which is 30-40 times more potent than cortisone and has fewer side effects.

Biologicals : Human blood plasma and plasma fractions have long been the subjects of Merck researches. In the preparation of gamma globulin, serum albumin and fibrinogen they played a very useful role. *Properdin*, a component of human plasma, is under investigation for its effect on cancer. Vaccines for polio, pertussis, typhoid and paratyphoid, small-pox, influenza, rabies, cholera, and common cold are other biologicals in which the Company is interested. In the field of sera and antivenins it is exclusive producer of the black widow spider antivenom.

Cardiovascular Drugs : Research in this field was stimulated in the 1940's when probenecid (*Benemid* Merck) proved of value in suppressing uric acid retention which causes gout. Merck laboratories, after ten years of research introduced chlorothiazide (*Diuril*) in 1957, which is effective not only as a diuretic but also in the treatment of hypertension, various oedema, nephrotic syndrome in children, liver cirrhosis, toxaemia of pregnancy, etc.

Veterinary and Agricultural Fields : Merck scientists have pioneered the application of vitamins, hormones, antibiotics and sulpha drugs in the feeding and treatment of animals. Some outstanding examples are Streptomycin for swine enteritis and calf scours ; sulphaquinoxaline, nicarbazin and glycarbylamide for coccidiosis in poultry ; and nithiazide announced in 1957, for turkey hexametiasis and blackhead of poultry.

In plant disease control *Agri-strep* (Merck), a Streptomycin formulation, helps control of diseases of peas, apples, tobacco, etc. Gibberellins, which are growth stimulants of plants and breaks dormancy in seeds, are now produced by Merck on a commercial scale.

Industrial Chemicals : This is a field of traditional interest with Mercks. Recent developments include ultra pure silicon which has varied uses in the electric and electronic industry. Other chemicals and derivatives for use in the plastic industries, are also under investigation.

RESEARCH ORGANIZATION

Fundamental Research : This group concentrates on basic researches in the medicinal and nutritional fields including the discovery of new antibiotics and vitamins.

Biological Research : This department is responsible for testing and establishing pharmacological properties and uses of drugs and chemicals.

Medical Research : Medical Research physicians determine the effectiveness and safety of medicinals administered to human beings. Products developed at Merck as

well as those brought to them are tested. The division also collects and publishes technical data for professional promotion of medicinal products. The physicians famous vade mecum, the *Merck Manual*, is also a result of such activity.

Animal Science Research : Researches in this division are comparable to those of the medical research group but covers animal health and nutrition. Large scale field trials of veterinary medicines are also the responsibility of this division.

Development Research : The diversified activity of this group covers process developments at laboratory and pilot plant levels, to evolve better forms of chemicals and drugs through the most practical and economic methods.

Virus and Tissue Culture : Researches in this area are directed to the development of methods for the prevention and treatment of viral infections.

Industrial Research : Devoted to the development of chemicals for industrial, electronic and agricultural fields.

ENGINEERING FOR PRODUCTION

Procedures for the large scale production of the products of research which show sufficient promise and usefulness, are first developed on a pilot plant scale, *i.e.*, 10 to 100 times that of the laboratory. Together with the engineering development laboratories the pilot plant steers the course from the laboratory to factory scale production. This phase permits process improvements by research chemists and engineers and provides data essential for design engineers to adapt existing facilities or to design and install new equipment for full scale production. As the process is developed, chemical engineers prepare flow sheets, layouts and specifications for equipment and furnish basic information on capital and manufacturing costs, data on sources of raw materials, plant location, waste disposal, economics of services required, and future expansion potential. Concurrently industrial engineers work on effective methods for the utilization of building and personnel, set up standards of productivity, design special equipment or make necessary adaptations of standard equipment such as those for materials handling, packaging, etc. Design and construction engineers then prepare final capital estimates on the basis of information, data, drawing, etc., provided by the other engineering sections. They also direct the construction and installation work. Thus engineering a product to plant scale requires the co-ordinated efforts of chemical, mechanical, electrical, industrial and construction engineers.

QUALITY CONTROL

The world wide reputation of the uniformly high quality of Merck products is the result of rigid quality control standards maintained by the company. Quality control extends to containers in which the products are sold, to storage conditions and raw materials. Materials are not released for distribution unless the chemical and pharmaceutical control staff have certified that they meet the standards laid down. From time to time the analytical research group develop new standards and new methods of testing and analysis. To handle the varied activities of quality control the division includes analytical, organic and inorganic chemists, microbiologists, physical and pharmaceutical chemists.

Merck's products now number over a thousand. Through manufacturing plants or distribution centres in more than 75 countries these products are available abroad as readily as in the United States. Licensees in several countries use Merck's processes and technical know-how. Among Merck's recent contributions to the development of the Indian pharmaceutical and chemical industry may be mentioned the technical assistance agreement with Hindus-

tan Antibiotics for manufacture of Streptomycin and Tata-Merck co-operative ventures.

Merck's objectives to serve as guide posts in facing the challenges and opportunities ahead, include :

"Maintain an environment within the Company that stimulates the personal development of its employees, recognizing that the continued prosperity and growth of the Company depend upon the character, ability and personality of the people who are associated with it. In that environment the dignity and rights of individuals are fully recognized, as is the importance of co-operation for the common good.

To maintain the kind of environment, it is essential to select for key positions men and women who appreciate its importance and are prepared to ensure its continued existence."

"Recognizing that research is the key to growth, support and manage their scientific activities so that they will continue to contribute effectively to the future development of the Company."

"Maintain high standards of leadership and a reputation for quality of people, products, research, production and marketing."

With a distinguished tradition and firm foundation in the field of pharmaceuticals and medicinal chemicals, the House of Merck builds for the future—for better health and better living for all mankind.

INSTITUTION OF CHEMISTS (INDIA) ASSOCIATESHIP EXAMINATION 1961

The Eleventh Associateship Examination of the Institution of Chemists (India) will be held in November, 1961. The last date for Registration is 30th November, 1960. The Examination in Group A (Analytical Chemistry) is divided into the following ten Sections and each candidate will be examined in two of them according to his choice as approved by the Council, in addition to the General Chemistry including Organic, Inorganic, Physical and Applied Analytical Chemistry — (1) Analysis of Minerals, Silicates, Ores and Alloys; (2) Analysis of Drugs and Pharmaceuticals; (3) Analysis of Foods; (4) Analysis of Water and Sewage; (5) Biochemical Analysis; (6) Analysis of Oils, Fats and Soaps; (7) Fuel and Gas Analysis; (8) Analysis of Soils and Fertilisers; (9) Analysis connected with Forensic Chemistry; and (10) Analysis connected with Leather Chemistry.

The Examination is recognised by the Government of India as equivalent to M. Sc. in Chemistry for purposes of recruitment of Chemists.

Further enquiries may be made to the Honorary Secretaries, Institution of Chemists (India), Chemical Department, Medical College, Calcutta-12.

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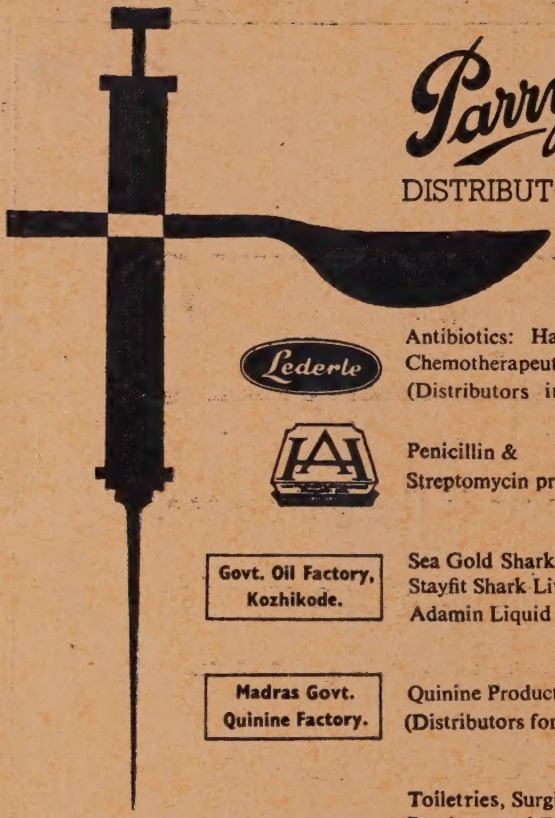

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